

STUDIES UPON CHRONIC BOVINE HAEMATURIA  
WITH SPECIAL REFERENCE TO THE  
ASPERGILLUS FLAVUS.

Thesis for D.Sc.

by

Satchidananda Datta, B.Sc. (Calcutta),  
M.R.C.V.S., D.T.V.M. (Edinburgh).

From the Pathology Department

&

Mycology Department  
University of Edinburgh.

1939.



# STUDIES UPON CHRONIC BOVINE HAEMATURIA

## WITH SPECIAL REFERENCE TO THE

### ASPERGILLUS FLAVUS SERIES.

The Thesis has been divided into two major sections: (I) The present position, and (II) The present investigation, and contains a Preface on veterinary problems in India.

In support of the Thesis the writer submits some of his published work, and provides a list of his miscellaneous other publications. Among the published work are to be found the results of the writer's original researches in other directions, notably upon a group of common, but hitherto obscure animal diseases of India, the helminthic etiology of each of which was first demonstrated by the writer and later confirmed by others. The arrangement therefore is as follows:-

#### Thesis.

Preface. The Nature and Significance of Veterinary Problems in Ancient and Modern India.

Studies upon Chronic Bovine Haematuria with special reference to the Aspergillus flavus series.

#### Original Publications.

##### (a) Mycotic Disease

A New Fungus, *Eidamella actoni*, parasitic to the Dog. Ind. Vet. Jl., 1932, 8, 1-10, Plates I & II.

##### (b) Helminthic Diseases

(1) New Researches on some Helminthic Diseases of India. XIIIth International Veterinary Congress. Zurich-Interlaken, 1938.



(2) The Etiology of Bovine Nasal Granuloma.  
Ind. Jl. Vet. Sc. & Ani. Husb., 1932, 2, 131-140,  
 Plates IX-XI.

(3) Schistosoma indicum, Montgomery, 1906, as  
 the Cause of a persistent debility in Equines in  
 India with a description of the Lesions. Ibid, 1933,  
3, 1-28, Plates I-VI.

(4) The Etiology of Bursati. Ibid, 3, 217-236,  
 Plates XIV-XIX.

(5) Microfilarial Pityriasis in Equines.  
 (Lichen tropicus). Vet. Jl., 1939, 95, 213-222,  
 Plates I-II.

#### List of Miscellaneous Publications.

(1) An Unusual Case of Chronic Rinderpest with  
 special reference to the Carrier Problem in this  
 Disease (in collaboration with V.R. Rajagopalan)  
Ind. Jl. Vet. Sc. Ani. Husb., 1932, 2, pp. 131-140.

(2) The Diagnosis and Treatment of Sterility in  
 the Stallion and the Bull. Ibid, 1933, 3, 185-197.

(3) Congenital Blindness in Calves. Ibid, 1933,  
3, 254-270.

(4) Prevalence of Tuberculosis among Cattle in  
 India. Ind. Vet. Jl., 1935, 12.

(5) Effects of Sterility and Impotence on Breeding Operations in India and Methods for their Diagnosis and Control. Ibid, 1935, 2.

(6) The Position of Tuberculosis and Johne's Disease in India with Suggestions for the Best Methods to Adopt for their Diagnosis and Control. Ibid, 1934, 11.

\* (7) Johne's Disease and Tuberculosis. World's Tenth Dairy Congress, Rome-Milan, 1934.

(8) The Etiology of Enzootic Bovine Haematuria, Part I. Ind. Jl. Vet. Sc. Ani. Husb., 1934, 4, pp. 341-361.

\* (9) "Khoojlee" or Lichen tropicus in Horses. Horse Breeding, Jl. of National Horse Breeding and Show Society of India. April, 1936, 57-58.

(10) Animal Diseases in relation to the Economy of Man and India. Agri. & Livestock in India, 1938, Vol. 8.

(11) How Far has the Study of Animals and their Diseases Influenced the Progress of Medical Research? Vet. Jl., 1939, 95, 25-31.

\* (12) To Review the Position of Bovine Mastitis in India and to Suggest Methods for its Diagnosis and Control. Proc. II Animal Husbandry Wing Meeting of the Board of Agriculture in India, Madras, 1936.

\* (13) To Discuss the Practicability of adopting Artificial Insemination in India. Ibid.

\* (14) Annual Reports of the Pathology Section of the Imperial Vet. Research Institute, Mukteswar, 1932-1937.

\* (15) Annual Report of the Protozoological Section of the Imperial Veterinary Research Institute, Mukteswar, 1938.

\* Publications marked with astericks have not been submitted.

## CHRONIC BOVINE HAEMATURIA

Pages

Preface

1-16

### Section I. The Present Position.

I. <u>History of the Disease.</u>	17-19
II. <u>Various Names.</u>	19-20
III. <u>Distribution of the Disease.</u>	22-31
IV. <u>The Importance of the Environment of Haematuria</u> <u>Farms and Localities.</u>	31-37
(i) Vegetation.	37-43
(ii) Soils.	43-45
(iii) Altitude.	46-48
(iv) Climate and season.	48-49
(v) Water supply and drainage.	49-51
(vi) Geology.	52-53
V. <u>Theories of Causation.</u>	54-73

### Section II. The Present Investigation.

I. <u>Introduction.</u>	74-83
II. <u>The Aims and Scope.</u>	84-89
III. <u>The Disease in India.</u>	90-98
(a) Actual localities affected.	98-106
(b) Incidence.	106-113
Seasonal.	
Age.	
Yearly.	
Sex.	
Incidence in the herd.	

	Pages
(c) Species of animals affected.	
(d) "Incubation period" of the disease.	
IV(a). <u>Clinical Symptoms.</u>	114-122
(b). <u>Clinical and Chemical Studies.</u>	123-150
V. <u>The Lesions.</u>	
(a) Pathological anatomy.	151-171
Macroscopic lesions.	
(b) Histopathological studies.	172-244
(i) Materials and methods.	172-191
(ii) Description of the lesions.	192-244
VI. <u>Haematuria Transmission Experiments.</u>	
(i) Position in literature.	245-257
(ii) Present studies.	257-277
(a) Small animals.	265-268
(b) Large animals.	269-274
(c) Experimental lesions.	274-277
VII. <u>Cultural Studies.</u>	278-358
(a) Materials and methods	{ of previous workers
	{ of present studies.
(b) The parasite -- twelve cultures.	304-313
(c) Mycological studies.	313-358
Cultivation on standard media.	317-325
Morphology.	333-346
Identification.	347-358
VIII. <u>Aspergillosis.</u>	
(a) Previous literature and general resumé.	359-374
(b) Transmission experiments.	376-383
(c) Histological studies.	383-390



IX.	<u>Fungi as Etiological Agents of Animal Disease.</u>	
	Discussion.	391-401
X.	<u>Experimental Studies on Treatment.</u>	402-414
	Methods of treatment and control to be recommended.	
XI	<u>Results of the Present Investigation.</u>	
	Discussion.	415-440
XII.	<u>Conclusion.</u>	441-447
	<u>References.</u>	i - xxv
	<u>Appendix</u> (a) Statement.	xxvi - xxviii
	(b) Methods.	xxix - xxxv
	(c) Records of experimental animals.	xxxvi - li

## PREFACE

### The Nature and Significance of Veterinary Problems in Ancient and Modern India.

Before dealing with any of the specific pieces of research upon which the writer has been engaged, it will be a helpful course to survey, even though very briefly, the development of Veterinary Science in India. This course is indicated, not only to justify the time and thought that have been bestowed on the present investigations, but what is more important, to provide the much needed perspective for the future growth and progress of veterinary work in India. In an art that was not long ago a close analogue of farriery, and in a field in which the introduction of a scientific atmosphere is only recent, the existence of the inertia, born of apathy, is not extraordinary. The chief obstacle to progress is still the lack of perspective and the want of an outlook regarding the function and ways of serving the community, for which the profession is designed. The unpleasant history of wasted time and endeavour that has been the result of unimaginative efforts of individuals lacking in the appreciation of the life and requirements of a needy community,

which should be their mission to serve, need not be recounted here. It will be sufficient to realise that a true insight into the requirements of a country in the light of the culture of its people is as indispensable in veterinary work, as in any other branch of social service aiming at the production of the lasting good of the community. Besides, when problems are viewed in relation to the past and the prospects of the future, they become so real and engender such sustaining interest in the execution of the many essential details of everyday work, which would otherwise certainly degenerate to trivialities of no consequence.

No attempt has yet been made to write up the complete past veterinary history of India, though very recently some attention to the British era has been exhibited. It is high time that a start was made, however small, by surveying the nature and significance of the veterinary problems of that country, starting from the earliest beginning.

Of all the many-sided problems that India, both ancient and modern, has had to face, none have been more fundamental or more extensive than those of her rural economy. Since time immemorial the cultivator and his cattle have formed the ever important bedrock of national life. Considerable changes in

outlook and life have no doubt taken place through the milleniums of Indian history, but the edict in the Rig Veda (iii, 8): "Let the bullock carry the load and the cultivators till the ground; let the plough cut up the earth well," stands as true to India today as it did in 4000 B.C. (Prof. Jacob), when that earliest chronicle of human history was written.

The state of culture and civilisation of the early Hindus of the era of what was then called Aryavarta, the home of the Aryans, appears to have been of a high order. The ancient culture is reflected in the elaborate systems of philosophy, the Vedas, the pioneering work in several branches of knowledge, including mathematical sciences and astronomy, Panini's grammar, Manu's code of laws, and the systems of Medicine and Surgery appertaining to man and animals. Orientalists have shown that works on Hindu Medicine were known and appreciated by other nations, particularly by the Arabs and Romans who quoted them in their languages. (Deitz, Jacolliot, Bhagawatsingh, 1896). Judged from the present standards, the ancient Indian chronicles contain much that is sound and valuable, while matters like demonology, exorcisms and magic conjurations were no doubt primitive. A consideration

of the extent of their knowledge is very striking. What is of particular interest to us in this survey, is that their methods of investigation were different from those of today. Their powers of introspection were highly developed: the ancients possessed in their jogic and metaphysical practices some apparently superhuman powers which they brought to bear upon their everyday problems. Records show that elephants were trained and richly caparisoned horses were used. The needs of the mere flesh received no less attention than those of the spirit. In short, the high degree of perfection attained was manifest in all branches of their activity.

Like other branches of knowledge, Hindu veterinary science grew on the matrix provided by the philosophy, represented by the sign of the Indian Swastika, emanating its fervour of peace and good will towards the whole creation. For instance, men and animals were deemed to have an identical destiny. Animal medicine, if such a term can be used, was held in equal honour to human medicine, and both grew together in close association. In Hindu prayers, animals received a prior mention to men, and in the laws of Manu (800 B.C.) animals were equally protected as men. That the cruel trappers and hunters of animals were not to receive



medical assistance was the injunction of Sushruta, the greatest of all Indian physicians. This veneration for animal life was further strengthened by Buddhism. King Asoka (270-233 B.C.) forbade the killing of animals, and established hospitals for men and animals alike throughout the country.

India still remains the only country in the whole world to provide sanctuaries (Pinjrapoles) for old and decrepit animals, while such animals are slaughtered for what they are worth in other countries. This unique feature of Indian thought still persists in spite of the ravages of time. A lot of the Hindu veterinary literature has been lost. The Brahmans had the custody of most of the literature, and were reluctant to allow it to go outside of their own circle. Evidence, however, exists to show that fractures and dislocations could be set, dissections on dead animals were practised, and the Hindus possessed a fair knowledge of the anatomy of the goat, sheep, horse and other animals. Surgical methods of castration, bleeding, cauterisation and blistering, etc., were practised and strict rules of hygiene were enforced. Simple preparations of animal origin, calcined bones, milk, urine, etc., were used in treatment. The action of a large number of herbal drugs and of calcined metals (Lauha bhasma,

calcined iron) were known. Reference is found to the stimulating effect of the herb, Arundhati, on the lactation of cows, and to the varying effects of the milk of different species of animals on the healthy or diseased human body. Further, the ancients seem to have realised the effect of the pasture and the colour of samples of milk, as altering the nutritional qualities of cow's milk, a fact which has just recently come to be realised. Long before the time of the Ramayana and the Mahabharata, veterinary science appears to have been highly cultivated. Reference is found to experts in Veterinary Science (Nala, an ancestor of the Pandavas and Nakula, one of them), and attention was paid to a variety of subjects. Particulars are available regarding the treatment of worms and insects of cattle (Atharva-veda), methods of administering clisters to different species (Charaka Samhita), and regarding the care of cows in health and pregnancy (Parasara Samhita). Again, the details of the two forms of tuberculosis (slow wasting fever and a pulmonary form) in elephants, and the varied prescription to be employed in such cases furnished in Hatyayurveda, elephantology, are remarkable. The fact that the ancients called Medical Science, Ayurveda (the science of life), and applied the same word to

veterinary science relative to the horse, the elephant and the like, bespeaks a comprehension not only of the unity of the healing science, but of what is being strongly urged today, that veterinarians should be experts in the comprehensive subject of animal health, rather than await the incidence of disease for practising the art and science of veterinary medicine and surgery. Mills, in 1838, discovered an old treatise which showed that in that far-off era, bovine diseases were classified and treated, and that rinderpest, anthrax, staggers, dysentery, piroplasmosis, meningo-encephalitis and ague must have been known (Lechlainche, 1936). While in Europe the term Hexapoda was coined by Latrielle in 1825, the name Satpada (six-legged) was used in the first century A.D.

The days of glory continued<sup>to</sup><sub>A</sub> about the tenth century A.D., when as a result of foreign invasion a process of disruption and decay set in. Invasion followed invasion and degeneration became more marked, bringing the narrative to the advent of the British into India in the eighteenth century. The East India Company started some horsebreeding farms in 1774, in order to meet the requirements of the Army, but as disease was rampant, these operations proved unsuccessful. In 1788 a Sanscrit work on

horse diseases was translated into English by Joseph Earles, and a similar treatise was published by Pigott in Calcutta shortly afterwards. Exercised as the Army authorities were over the question of disease among horses in 1793, there was not a single trained man in India and only two in Britain who could be asked to help. It was not until 1799 that the services of a few trained veterinary officers could be secured, but little is known of what these men were able to do. William Moorcroft, however, was different. He was appointed in 1808 to suppress disease among horses at a salary of £3000 a year. After a medical career at the Liverpool Infirmary, Moorcroft received training at the Veterinary School at Lyons, and left a profound impression on veterinary problems of the day. He found Bursati very prevalent at Pusa, and he drew pointed attention to glanders, strangles, paraplegia and particularly to a fatal disease since then identified as anthrax. Bursati affects horses, and was known to the local people as somehow connected with Bursat, rainy season, and with flies. Moorcroft considered Bursati as infectious, and made some valuable observations on acute and fatal cases of equine paraplegia. Since his early death in 1825, nothing noteworthy seems to have happened till the appointment of Hallen as



staff veterinary surgeon in 1850. Hitherto, the requirements of the Army was the only reason for which the East India Company employed men for veterinary work, and most of these were unqualified men. A new orientation to veterinary work was given by Hallen for he was a very outstanding man, full of initiative and endowed with a rare understanding of the practical needs of the country. Hallen visualised for the first time large scale and first class establishments for training the agricultural classes of India, and proposed machinery for organised livestock work. He established an Army veterinary school at Poona in 1862, and a civil veterinary school at Babugarh in 1877, which was later moved to Lahore to become the present Punjab Veterinary College. He was appointed the President of the Indian Cattle Plague Commission, which among other things recommended the establishment of a Veterinary College at Calcutta. A civil Veterinary Department was established in 1889, with eighteen officers transferred from the Army Veterinary Department, and it was placed under Hallen in 1892 who became its Inspector General. Hallen's work had already stimulated interest in veterinary work, and several officers actively participated in investigating various obscure diseases. Bursati and other equine



diseases, like Lichen tropicus, calcareous nodules in internal organs of debilitated horses\*, anthrax, worm infestations, paraplegia and Kumri received considerable attention from several workers, including Queripel, Frederick Smith (later made a knight). The most outstanding scientific discovery of the time was the demonstration in 1881 by Griffith Evans of Trypanosoma evansi, the causative agent of equine surra. This discovery was important not only to India, where Surra was a very troublesome condition to deal with, but also to the progress of scientific research generally, as opening up the study of other protozoan diseases of both man and animals. Veterinary journalism in India may be said to have started in 1882 with the appearance of the Quarterly Journal of Veterinary Science in India under the editorship of Steele, who became the first Principal of the Veterinary College at Bombay when it was established in 1886. The Journal was in existence for eight years and contributed a great deal to the progress of the profession. The next important landmark was the appointment by the Government of India of Alfred Lingard, a Welsh medical man, as an Imperial Bacteriologist in 1890 to investigate animal diseases all over India, and to evolve by biological research means for the prevention

\*The Etiology of these were elucidated by the writer.

and cure of epizootic diseases. The necessary laboratory accommodation was provided for him at the Science College, Poona, till 1893, when at the suggestion of Lingard the laboratory was moved to the present situation at Mukteswar in the foothills of the Himalayas. It may be mentioned here that the Mukteswar Institute has the distinction of being the oldest scientific research institute to be established in India. The Veterinary College at Calcutta was brought into being in 1894, and, like the Bombay College, largely as a result of generous gifts of land and money by two Indian philanthropists. At the invitation of the Government of India, the celebrated German bacteriologist, Robert Koch, came to India in 1897, and demonstrated at the Imperial Bacteriological Laboratory, as the Mukteswar Institute was then called, to a group of veterinary officers from different parts of India, the method of Rinderpest immunisation which had already been successfully adopted in South Africa. The production of potent anti-serum against Rinderpest was thus achieved in 1899, and the anti-sera against anthrax and haemorrhagic septicaemia in 1901. In the latter year, the first provincial veterinary department to deal with disease and cattle breeding problems was set up in the Punjab. This pre-eminence in

organisation has been maintained to this day, and the Punjab has the largest veterinary staff of all provinces, administering besides disease control, extensive animal breeding operations which are denied to the profession in other provinces. Initially, veterinary officers for civil work used to be drafted from the Army, but direct recruitment was started about 1902 with five men, of whom three are worthy of mention, viz. Stewart Stockman, Montgomery and Jethiji. Stockman (later Sir) rose to be the head of the department at the British Ministry of Agriculture, while Montgomery's systematic work on the Schistosoma group of helminths, carried out at Mukteswar, has become classical, and later he rose to be the Veterinary Adviser to the Colonial Office in London. Jethiji was a very rich nobleman of the Central Provinces, and happened to be the first Indian to obtain British qualifications. He appeared to be highly promising, but unfortunately he died within the first few years of his service in rather obscure circumstances. The Madras Veterinary College was established in 1904, and the most recent addition has been the Veterinary College at Patna, in Bihar, in 1930. The Journal of Tropical Veterinary Science was started by Montgomery, Baldrey and Pease in 1906, but with the abolition of

the post of Inspector General in 1912, the Journal ceased publication. Since 1908 veterinary officers had held periodical conferences and discussed matters of research, education, publications and cattle industry.

Slow progress continued with slight increase in staff and in monetary grants, and in the provision of veterinary hospitals and dispensaries. In 1926 a Royal Commission was set up to investigate and report on the state of agriculture and rural economy, and to make recommendations for the promotion of the welfare and prosperity of the rural population. The Commission discovered that the provision for veterinary aid was totally inadequate, and a substantial increase of staff of all grades, at the rate of one veterinary assistant surgeon for every 25,000 cattle and a gazetted officer for each district with about 600,000 cattle, was recommended. Further, to develop and co-ordinate agricultural and veterinary research, an Imperial Council of Agricultural Research was recommended, which came into being in 1929. The function of the Council is to develop and co-ordinate agricultural and veterinary research, and already schemes of animal husbandry research costing £127,500 have been financed. Disease investigation officers have been provided by the Council for every



province. At one time a single man, the Imperial Bacteriologist was expected to engage himself in solving all the veterinary problems of the whole country. The same function is now served at the Mukteswar Institute by the different composite sections of Pathology, Serology, Protozoology, Biological Products, Poultry Research and Animal Nutrition, with separate specialist staff for Acidfast Infections and for Contagious Abortion in Cattle.

Arrangement is now being made for the addition of an Animal Genetics Section. Each of these Research Sections is under the direction and supervision of a Senior Research Officer who is individually responsible to the administrative head, the Director of the Institute.

Thus, all the fundamental research is carried out at the Central Institute, and results of economic value have emerged. Recently a cheap and efficient tissue vaccine against the most dreaded Rinderpest has been elaborated. This is being produced from goats on the spot and extensively used all over the country. The results of successful research, including any biological products that are evolved by the Institute, are applied in the field by the provincial departments. The Institute maintains a close contact with officers in the provinces, by the



interchange of visits, postgraduate classes, periodical courses, publications and regular official correspondence. A certain amount of research work has also been carried out in the laboratories attached to the various provincial veterinary colleges, by those who took a specially live interest in their work, and here the names of Raymond at Calcutta, Krishnamurti Ayyar and Rao in Madras, are noteworthy.

The above is in outline the organisation that exists now in India for the study of problems affecting animal health and productivity. Numerous baffling problems exercise the veterinary workers in India today. In 1892 Burke estimated "after the most careful investigation, that the annual money loss to India alone from preventible contagious disease among agricultural animals, cannot be less than £6,000,000 sterling." It may be added that as a result of one disease alone, the Warble fly infection, the depreciation in the value of hides in India has recently been estimated to be £11,250,000 annually. The substantially large export trade in livestock and livestock products enjoyed by the country, has rapidly dwindled down. For instance, while the return for 1933-34 showed an income of £24,225,000, that of the following year was only £9,675,000. All this loss sustained is ascribable

to the incidence of disease. The Indian Zebu cattle has been found to be the most suitable animal for the tropical and subtropical countries, and if the risk of introducing disease with the cattle could be definitely eliminated, the large overseas market could be regained. Regarding the actual size and potentiality of the cattle industry, it must be added that India possesses the largest cattle population of any country in the world with 215 millions, while the countries next in order are the U.S.S.R. with 65 millions, and the U.S.A. with 58 millions. A recent estimate shows that the total annual cash value of the stupendous cattle industry of India, including dairy products, labour rendered to stock owners, and manures is about £975,000,000. Further, cattle mean much more in India than elsewhere since she is directly dependent upon cattle as the motive power of agriculture, for the transport of marketable goods, for supplying nutrition to a largely vegetarian population, for the fertility of the soil and for the raw materials for the tanning and other industries. Thus cattle form the basic key to the whole economic structure and rural welfare, and the immensity of the task before the much neglected veterinary services is clear.

PART I

STUDIES UPON CHRONIC BOVINE HAEMATURIA  
WITH SPECIAL REFERENCE TO THE  
ASPERGILLUS FLAVUS SERIES

I. HISTORY OF THE DISEASE.

Although a large volume of literature on the disease has accumulated in different languages, a considerable number of workers appear to have paid little attention to publications from outside their immediate jurisdiction. Consequently, there have been too many unnecessary repetitions and comparatively little attempt to correlate investigations or to break new ground. As far as the English veterinary literature is concerned, and this, according to the writer's studies, applies to that in other languages, Thomas Topham, in 1787, would appear to be the first to publish a general, though quite a lengthy, discourse on haematuria of cattle. Credit has been given in some continental literature to Pichon and Sinoir for being the first to introduce the name haematuria, in 1863 and 1864 respectively. It appears that a part of the credit should legitimately go to Topham. Again, contrary to the impression existing among certain authorities that the history of the literature on bovine haematuria starts with 1863 or 1878, it may be mentioned that the available evidence clearly shows that the disease has been recognised in more than one country in Europe for at least a century.

Chronic bovine haematuria or, better still, enzootic haematuria of cattle, presents a clear-cut and precise syndrome, and truly the term haematuria has acquired a special significance in veterinary literature. Haematuria has been a serious scourge in cattle farms in many countries, and its diagnosis as a separate disease entity does not appear to have presented much difficulty. The earliest recorded cases traced so far relate to several European countries. Topham's discourse is dated 1787, but it is not possible to find out from his descriptions where he obtained all his sixty years' experience of the disease. Haematuria cases were diagnosed in the forties of the last century (Drouard, 1838, Anderson, 1842, Hubner, 1842, Vaes, 1843, Vigney, 1845, Raconnat, 1847). Outside of Europe, the earliest report would appear to be from Australia, made some fifty years ago (M'Caffrey, 1892), and the first report from Canada is dated 1907. As far as other countries are concerned, the disease was reported from Bulgaria in 1910, from Hawaii Islands in 1911, from East Africa in 1912, from Washington, U.S.A. in 1913, from Ireland in 1917, from England, Scotland and Wales certainly about 1924 (earlier reports from England probably exist), from India in 1921, from New Zealand in 1926, from Jamaica in 1932 and from Yugoslavia in 1935. Although it is not



possible to form an opinion as to whether the disease was ever introduced from one country into another, it may be advisable to note the above dates, as they may turn out to be suggestive, if in the future the disease is found to occur in a new locality in an affected country, or in an altogether new country having little or no traffic in cattle from known enzootic areas.

## II. VARIOUS NAMES.

The different names under which the disease has been described from time to time, in different countries, provide a good impression of what form the most outstanding general features of the malady. It will be of interest therefore to recount them below:-

Maladie de bois Holzkrankheit Moor Ill	(Law, 1901)
Blutharnen	(Hubner, 1842)
Stallroth Weidroth	(Hink, 1886)
Hematurie essentielle	(Detroye, 1891)
Ematuria enzootica	(De Filippis, 1891)
Cystite hemorrhagique	(Galtier, 1893)
Hematurie des bovides	(Boudeaud, 1894)

Hematurie chronique	(Delcroix, 1905, Lienaux, 1905)
Cystite verrucosa	(Goetz, 1906, Klobouk, 1931)
Haematuria vesicalis bovis rodopensis	(Angeloff, 1910)
Red Water	(Case, 1911, Kalkus, 1913)
Endemic Haematuria Illawater Redwater	(Cleland, 1911)
Bovine Haematuria	(Hadwen, 1912)
Cystite chronique hemorragique	(Cadeac, 1913)
Endemische Hematurie mit Blasentumor	(Ichikawa, 1921)
Urocystitis haemorrhagica	(Miyamoto, 1927)
Enzootic haematuria	(Dickinson & Bull, 1929)
Cystirrhagie	
Queensland Malady	
Cistite cronica emorragica vegante	(Giovine, 1933)



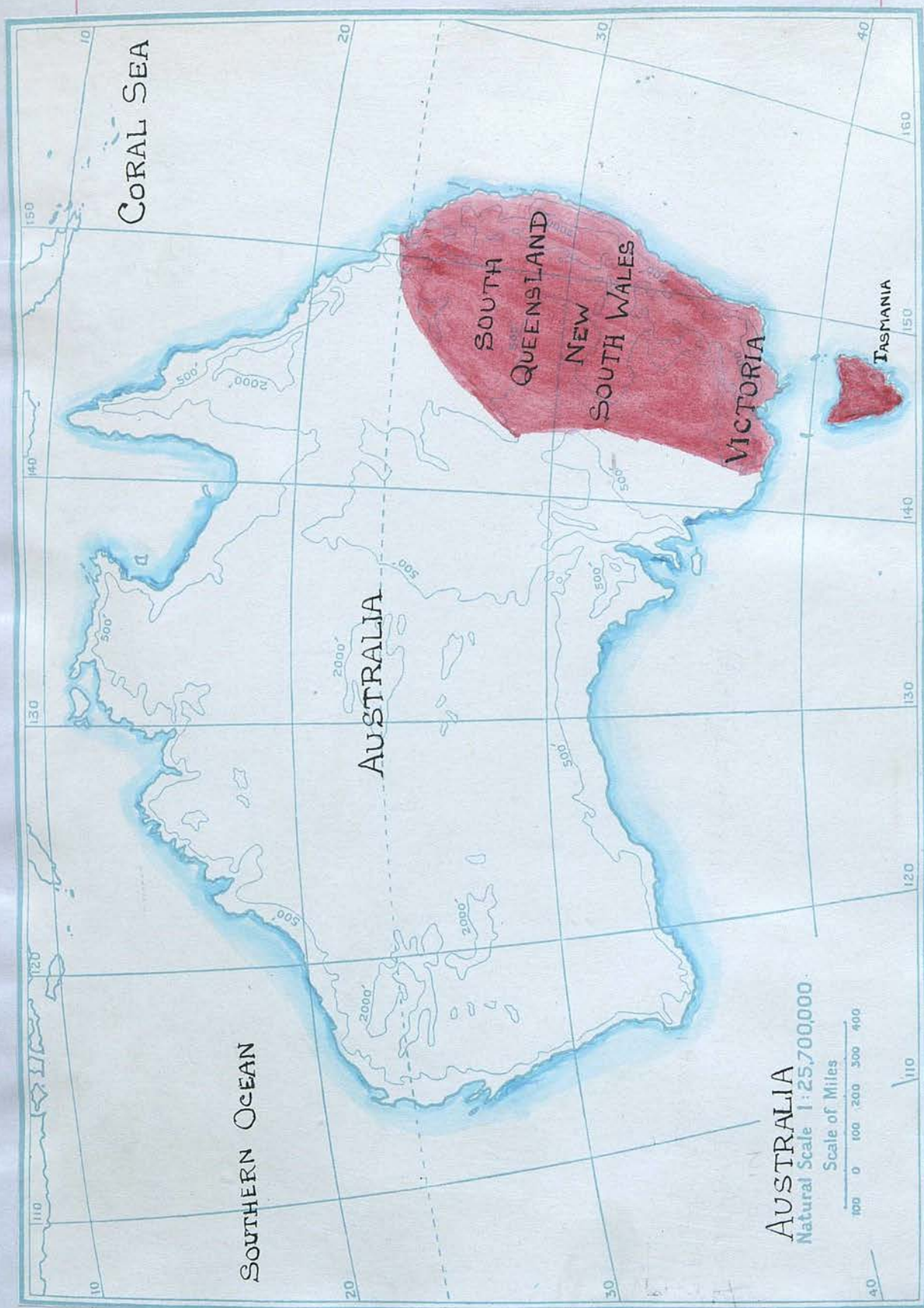




### III. DISTRIBUTION.

It will be shown below, that the disease occurs throughout the world, and the particular areas where it occurs are restricted and widely separated from each other. A comprehensive list of the enzootic areas, or a complete account of the geographical distribution, is not to be found anywhere in literature. It is proposed, therefore, to set together as much of the information as can be collected from original sources, and to prepare maps showing the world distribution. <sup>(p.21,23,25,27,29,97)</sup> In the future, other countries or areas may have to be added to the list, and it is to be hoped that with proper preventive methods being devised, areas may be rendered disease-free. According to the available information, the following countries are reported to have the disease within their boundaries:-

Austria	(Reisinger, 1924)
Australia	(M'Caffrey, 1892, Williams, 1894, Cleland, 1911)
Belgium	(Lienaux, 1919, Lahaye & Rulot, 1926)
British Columbia	(Bowhill, 1907, Rutherford, 1911, Hadwen, 1912)
Bulgaria	(Angeloff, 1910, Popow, 1936)
Czechoslovakia	(Klobouk, 1931)
England, Scotland and Wales	(Craig & Kehoe, 1925, Imp. Bur. Ani. Health, 1931)



Geographical Distribution  
of *Haematuria* in red.



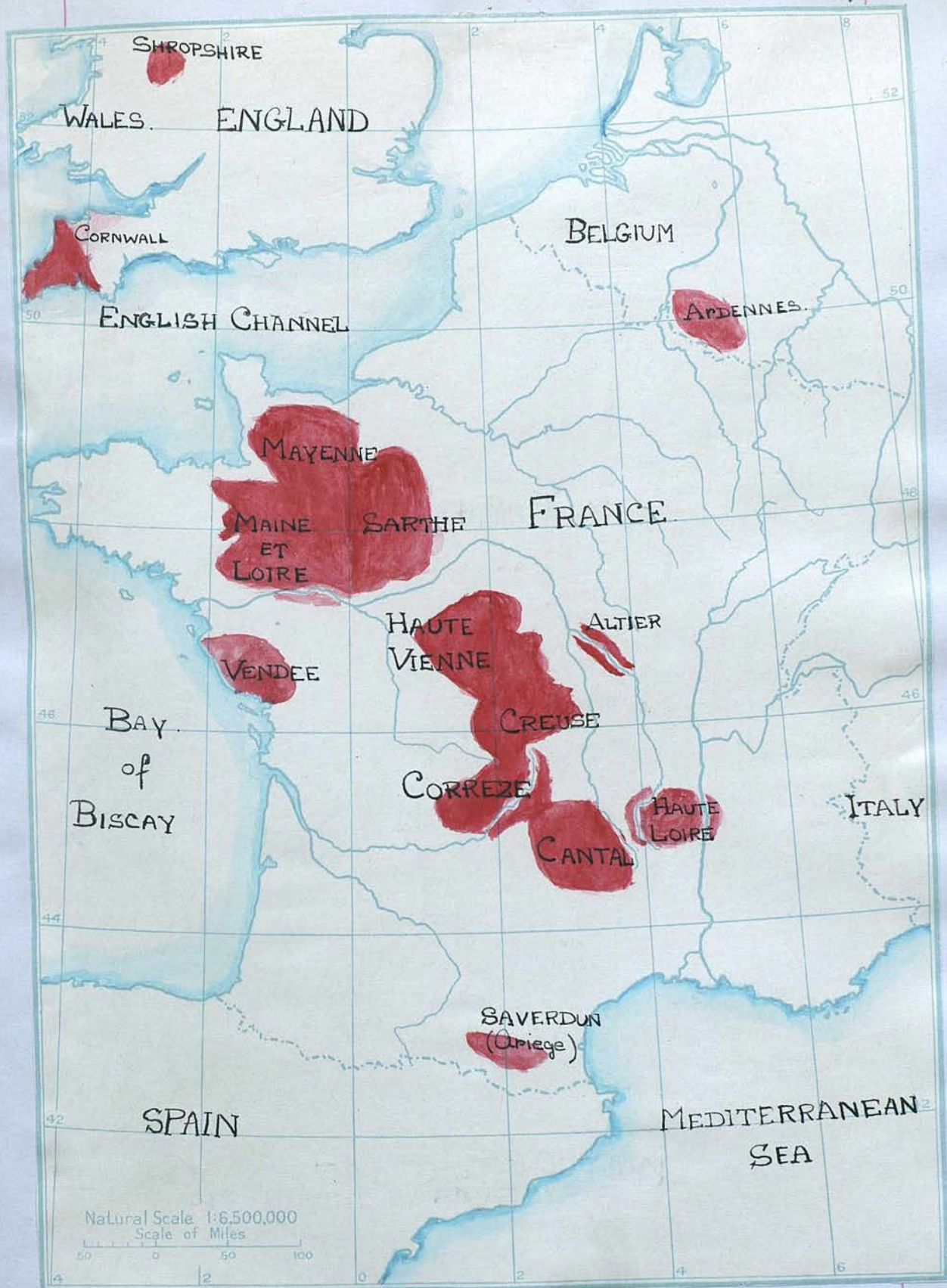
Formosa, Japan	(Ichikawa, 1921, Miyamoto, 1927)
France	(Avril, 1929, Pommeret, 1937)
Germany	(Ross, 1879, Arnold, 1890, Schlegel, 1934)
Hawaii and other Pacific Islands	(Case, 1911)
Hungary	(Hutyra & Marek, 1938)
India	(Rangaswamy, 1922, Datta, 1934)
Ireland	(Craig & Kehoe, 1923)
Italy	(Cavalazzi, 1889, Adami, 1917, Gottardi, 1935)
Jamaica	(Lockett, 1932)
Kenya Colony	(Kearney, 1918)
New Zealand	(Kerrigan, 1926)
Oregon	
Republic of Colombia	(Giovine, 1933)
South Africa	(Alexander, 1938)
Switzerland	(Goetz, 1906)
Washington	(Kalkus, 1913)
Yugoslavia	(Butozan, 1935 and 1938)

Regarding the actual localities where haematuria occurs in the above mentioned countries, exact information has not been recorded from one and every country. In the Commonwealth of Australia, the disease occurs practically throughout the south east, including the high lands of Victoria, Tasmania, New South Wales and Southern Queensland. In New Zealand,





the disease occurs in the west coast district of South Island, particularly in Murchison and Inanguhua. In North America, the disease occurs on the Pacific slope of the Cascade and Coast Ranges, extending from the Fraser Valley in British Columbia to Washington and Oregon in the United States of America. Considerable details regarding the affected areas in British Columbia are available. It occurs principally in Fraser Valley district, consisting of uplands bordering on the north and south sides of the Fraser River, encompassed on the west by Vancouver and by Hatzic on the east, in the north by the river and in the south-east as far as Rosedale, and in the south extending into the United States but not including the delta islands. The disease is recorded from the Municipalities of Surrey, Langley, Matsqui, Chilliwack, Maple Ridge and the Lilloet district, which are all in the above mentioned area, and within a distance of approximately seventy miles inland from the Pacific Coast. The disease has also been reported as occurring on Bowen, Galiano, Salt Springs and Vancouver Islands, which are all adjacent to the mainland of British Columbia. Definite evidence has been secured recently that it also occurs on the Terrace and East Kootenay districts, both of which are widely separated and considerable distances from the Fraser Valley. The possibility that the





environment responsible for the malady covers an even wider area in British Columbia has been suggested (Bankier, 1936).

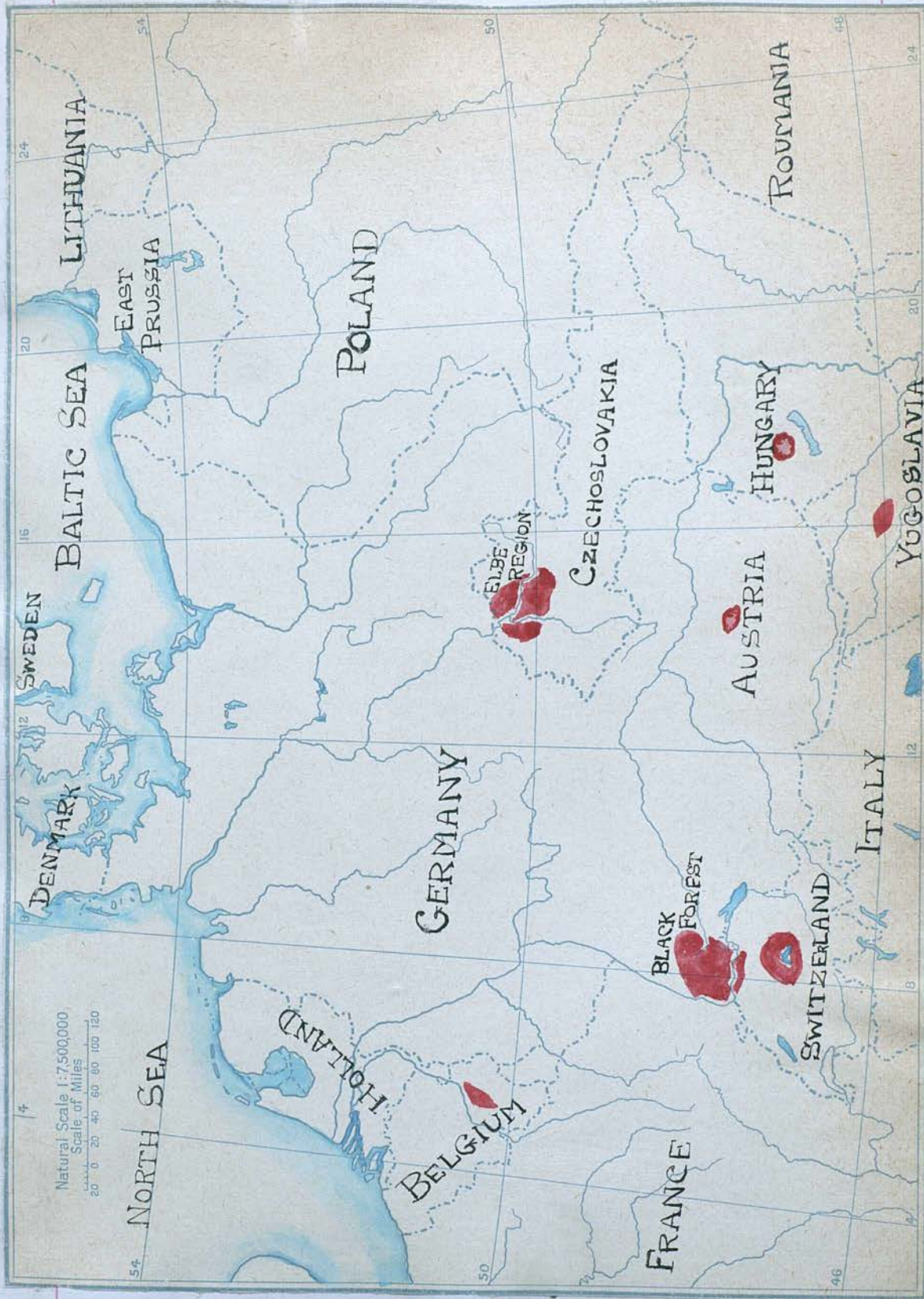
In the Pacific Islands, most cases have been reported from the Island of Hawaii, the principal infected districts being Kona and Hamakua. Some cases occur in the Island of Maui.

In Jamaica, most reports have come from Southern Manchester. Less frequently cases have occurred around Christiana, Lacovia and a limited area of the coast region of St. Ann.

In East Africa, the disease appears to be confined to the Limuru district.

In France, the disease occurs in the west and in the vast territory enclosing the central plateau. The disease is particularly common in low-lying areas and scarcely seen above a height of 2400 ft. It occurs in the Mayenne, the Sarthe, and spreads into the Maine et Loire and the Indre. It inflicts great losses in the Creuse, Allier, Vendee, Haut-Vienne, Cantal, Haut-Morvan, Saverdun (Ariege), the Correze and Haute-Loire districts. In Belgium, the disease occurs mainly in the Ardennes Mountains, but occasionally in Brabant, Beauvechain, Hammemille and Weed-Saint Georges. In Germany, cases occur in the south, in the Black Forest, among stall-confined cattle during and after a dry summer, and in cattle which





Haematuria areas in red.

are turned out on high-lying wooded pastures. The disease occurs in the Elbe region.

In Yugoslavia, the disease is found in all the counties (Banats), especially in the hilly and mountainous regions which are wooded. The disease affects animals in 95 out of 350 circles, the average for the whole country being 27.5 per cent. The percentage of land area affected in each circle has been worked out with the following results: Banat of Danube, 1.9 per cent; Banat of Morava, 5 per cent; Kustentland Banat, 18.1 per cent; Zeta Banat, 18.9 per cent; Vardar Banat, 44.9 per cent; Drava Banat, 48 per cent; Drina Banat, 62.4 per cent, and Vrbas Banat, 68-76 per cent.

In Switzerland, Goetz (1906) says that stallrot is known to occur somewhat commonly in certain regions. He studied the disease at Zurich, and some of his cases came from Benken, Burghalden, Baretswil and Willisan.

The disease occurs in some other countries in Europe, e.g. in Hungary, in the central provinces of Italy, in Czechoslovakia. Regarding the incidence in the British Isles, Wallis Hoare stated, in 1914, that as far as he was aware it had not been noted. The disease occurs in Cornwall and Shropshire in England (Downham, 1938), in Carnarvonshire and



Merionethshire in Wales (Roberts, 1930) and in the north of Scotland. In Ireland, it occurs in the Suck Valley in Co. Roscommon. The exact extent of the disease in these areas is not known, but it is believed that it may be widely distributed in Ireland. Upland farms are usually affected. According to some private information obtained from Mr. H.G. Lamont of the Veterinary Research Division of Northern Ireland, the disease exists in Belfast, and along the Antrim seacoast, and also in the counties of Down, Londonderry and Tyrone.

In some countries, the actual localities where the disease occurs have yet to be surveyed and clearly defined.

#### IV. THE ENVIRONMENT OF HAEMATURIA FARMS AND LOCALITIES.

It has been observed already that geographical distribution and enzooticity are two of the most striking features of the disease. Why is the disease so particular in occurring in special places and tending to remain localised there, without any obvious spread to the neighbouring areas? This question has been asked from the earliest times, and most workers have set out to ascertain the nature of

the inherent factors which appear to govern the phenomenon of distribution in such a marked way. Are the environmental factors merely chance associations, or have they some significance? Some workers have gone so far as to believe that the environment itself produces the disease, and stray observations supporting this belief have been recorded from time to time. Attempts have been made to define the localities accurately, and to determine by careful observation and scrutiny which of the factors are really the indispensable features. The general experience of workers, from the early date when the disease first forced itself on human attention, has been that haematuria localities provide conditions conforming more or less closely to an invariable pattern. By the process of elimination, repeated attempts have been made to determine the minimum optimum conditions that would allow the disease to appear in a farm or in an area. Comparisons have been made from different angles between infected and non-infected areas, but though an increasing amount of appreciation of the localities has no doubt resulted, the main question still remains to be answered.

It has been the general experience that the habitats of the disease are chiefly upland areas,

which are wooded and situated on both high and low mountains. Field observations have been recorded from both Mounts Gambier and Schank in Australia, to the effect that when paddocks which had not been ploughed for years (described as "stale and ferny") were leased, cases of haematuria started to appear in farms that had been free. After carefully weighing all the evidence obtained from farmers, Bull, Dickenson and Dann (1932) came to the following conclusions:- (i) That certain farms are definitely more likely to give rise to the disease. (ii) That a regular stream of cases does not always develop on any farm, but that after the disease has become manifest in several animals, a period of years may elapse before more cases develop. (iii) That within the haematuria area some farms never show active cases of the disease. Law (1901) states that it is the disease of woods and waste lands, of damp undrained lands, of lands rich in vegetable humus or vegetable moulds. In the country most recently added to the list of haematuria countries, Butozan (1938) has also observed that the disease is a malady of wooded hills or mountains.

Apparently contradictory reports have, however, been made to the effect that the disease occurs in the low-lying areas of France, and that the bench



lands of Canada, where haematuria is found, are only a few feet above the sea level. In Australia, Cleland (1911) noticed that the disease was confined to high lands, and Case (1911) observed the same thing about the incidence in Hawaii Islands. Detailed investigations of other features, like the character of the soil, water and vegetation of the areas, have been made. While the disease is normally a disease of pasture animals, it occurs in both stall-confined and grazing cattle in Germany. Moussu states that in all countries, bovine haematuria is a disease of certain poor regions of which the soil and flora present special characters. The idea of the influence of the soil, flora and action of certain plants, has been sustained everywhere.

Hadwen (1917) stated that the disease in Canada was associated with poor farm lands, woodland, newly cleared farms, or farms that were either neglected or falling out of cultivation. The disease has therefore been called "a poor man's disease." He added that the disease is due to a class of vegetation rather than individual or special plants. It is usually stated that with improved management, cultivation or top dressing of the land, the disease gradually disappears, but it is not clear what modification of the flora, or other transformation in

the land conditions, bring about the disappearance of the disease. Others factors associated with its occurrence are high rainfall, an open or light soil, and the presence of bracken fern. In the earliest reports from Australia, M'Caffrey states that in New South Wales the disease is more prevalent after a dry season where there has been a great rush of soft grass.

Certain field observations, recorded by Lienaux (1911) to emphasise the enzooticity of the disease, are worthy of note. For instance:-

(i) One farmer, who had always suffered from the existence of the disease in his large farm, disposed of the part that was wet and waterlogged, and thus restricted his own cattle rearing operations to the better part left for himself. He noticed that the disease altogether disappeared from his own farm, while much illness was exhibited in the other part taken over by a new farmer.

(ii) A farmer purchased a farm where the disease existed. He disinfected it and started out with a fresh batch of good animals, but he obtained fodder from the same source as the previous farm holder. The disease appeared again in the farm.

(iii) One farmer, who was always troubled with the disease, started obtaining the food supply of

his cattle from an area that was known to be free. After this, there was no disease among his cattle for one winter, but the following year the disease again manifested itself. Whether this was due to accidentally contaminated food, or due to the animals having had access to old food of the locality, was not clear.

(iv) Another farmer used to obtain food for cattle from an area outside his locality but could not get rid of the trouble from his herd.

The author went further to record that when affected animals were transferred from the Ardennes, the disease was "cured".

Contrary to the existing hypothesis incriminating either the vegetation or the soil type, Bankier (1936) asserts that these have no direct relationship to the disease whatsoever. His experience, however, confirms that the majority of haematuria farms have a limited amount of land under cultivation, the balance being bush or rough pasture. He observes that the disease appears to be confined to certain farms rather than any particular area. From the crucial experimentation under field conditions now in progress at Milner B.C., highly interesting results are promised. The positive evidence already collected, which need not be gone into here, tends to establish

a definite relationship between pasture conditions and the development of the disease. Literature shows how very difficult it has been to establish any correlation between environmental factors and the disease. The difficulties of controlled work under conditions existing in the field, have been great, but there is no doubt that the possibilities have been greatly narrowed down:-

(1) Vegetation.

The toxic effects of plants have long been suspected, and many individual plants, or groups of plants, have come into both prominence and disrepute in this connection. Young shoots of the oak, ash, privet, hornbeam, hazel, dog-berry, coniferae (Law, 1901), ferns, sedges, rushes, hellebore (Galtier), and many others have been included in the list of plants from which animals have to be protected and kept away. Botanists have co-operated to a large extent in these investigations carried out in several countries. In Ireland, it is stated, the fields to which haematuria cases have access, contain grasses which are for the most part coarse, and that the herbage contains moss, gorse, bracken, arum, iris and sedges. Plantago, Ranunculus, Rumex, Anemone, Carex, mosses and Colchicum were found



growing in and around haematuria farms in Belgium, and the pastures contained Hieracium, Auricula, Achillea, Hypericum tetrapterum, Hypochoeris radic. and Hypochoeris macul. (Lahaye & Rulot, 1926).

In British Columbia, the native and herbage growth consist principally of alder (Alnus glutinosa, Goertn.), fir (Abies balsamea, Mill.), birch (Betula occidentalis, Hook.), vine maple (Acer circinatum, Pursh), cedar (Thuja occidentalis L.), bracken (Pteris aquilina L.), willow (Salix sp.), hazel (Corylus americana, Walt.), broad-leaf maple (Acer glabrum T. & C.), hemlock (Tsuga canadensis L. var), and cotton wood (Populus deltoides, Marsh.) The prevailing cultivated pasture herbage consists of timothy (Phleum pratense), June grass or Canada blue grass (Poa compressa), white clover (Trifolium repens), red clover (Trifolium hybridum), orchard grass (Dactylis glomerata), perennial rye grass (Lolium perenne), red top (Agrostis alba), meadow fescue (Festuca elatior) and Yorkshire fog or velvet grass (Holcus lanatus). The plant communities dominant in the affected areas are 'fire or burn indicators', such as would be expected to occur on logged or burnt off lands, whereas the 'plant indicators', among the pasture plants are those usually associated with overgrazing. It is also to be



noted that the majority of herbage plants of the locality are acid-loving individuals. (Hill, King & Laird, 1933). Recent Australian workers have, however, failed to detect any difference in the herbage and grasses between affected and non-affected farms.

Of the plants growing in enzootic areas, Cleland (1911) in Australia became highly suspicious of Homalanthus populifolius (Euphorbiaceae), Indigofera australis and Goodia latifolia (both Papilionaceae), while Hadwen in Canada thought it necessary to experimentally test out the local plants, bleeding heart (Dicentra), deer grass (Achlys triphylla), alder, and bracken (Pteris aquilina). In New Zealand haematuria localities were covered with beech (Nothofagus sp.). The possibility of certain toxic plants causing haematuria has been tested by several workers, and reference to this work will be made later, in connection with the various attempts at experimental transmission of the disease.

One plant, however, more than any other requires to be considered here in some detail. The bracken fern (Pteris aquilina) has been prominently mentioned and observed as invariably associated with haematuria farms in all countries. For instance, Downham (1938) states that haematuria farms in Shropshire, in

England, are mostly hill farms with bracken in the pasture. The converse, however, does not hold true. The symptoms of bracken poisoning are well known and do not include the syndrome of haematuria, and some years ago Stockman studied the subject in England. Bracken has been called the insidious foe of the farmer, and the great increase of this plant in recent times in Great Britain has been causing some concern among recent writers. Bracken is no doubt a most widely distributed plant in temperate and tropical regions alike, much more widely spread than haematuria. According to a recent report, the cultural requirements of bracken are being studied in haematuria farms in Canada. This point has already been studied in England from the purely agricultural standpoint. Surveying the story of the bracken fern, Long & Fenton (1938) provide the following interesting details. It does not seem to flourish on calcareous soils or over limestone unless the carbonate has been washed out. It occurs in most rough grazings and in many old permanent pastures, in most open woodlands and on sites of formerly wooded areas. Bracken is a typical part of the flora of rather open Pedunculate Oak woods or forests, and also appears with the Sessile Oak, and in many other plant associations. A series of wet

seasons, that have probably been very favourable to the spread of the plant by spore growth, has been suggested as one of the causes of the recent increase of bracken in British pastures. It may be remarked in passing, that certain authorities have described the special features of haematuria localities in very similar terms.

Authorities have repeatedly stated that the disease is associated with upland farms where much of the land is badly cultivated or has gone out of cultivation. Such upland grazings are expected to become bracken infested. Similar other plant associations of haematuria exist. Attempts have been made to ascertain the common factors underlying the plant associations of haematuria, but no data capable of providing a satisfactory explanation have yet been obtained. Some exact data with regard to the vegetation of haematuria localities have, however, been put forward by Hill, King & Laird (1933) who stated that it had been definitely established that there was an inherent difference in the mineral and inorganic compositions of different species of plants. The leguminous plants usually contain a large excess of calcium over phosphorus, whereas there is generally an excess of phosphorus over calcium found in the graminous plants. Further, it has been found in

Canada that the calcium content of feeding stuffs grown on haematuria farms, is comparatively higher, with a wider range, than those produced on disease-free farms, and that the manganese content is distinctly lower in the majority of cases. The investigators think that the physiological effect of this slight deficiency may be of importance, as manganese is an essential element of plant growth which probably assists in animal nutrition. Similarly, Theiler (1929) emphasised that the remarkable coincidence of phosphorus and manganese deficiency in haematuria areas in Australia should not be lost sight of. The finding in Germany that the hay of haematuria localities is rich in crude fibre, but deficient in albumin and poor in mineral salts and vitamins (Schlegel, 1934), is perhaps not so interesting as the results obtained in Canada, but should not be altogether allowed to be lost sight of. Bankier (1936) says that the dry feed supplied to cattle in Milner B.C. does not appear to have any relationship to the production of the disease, nor is the bodily condition of animals a predisposing factor. In the opinion of Bull, Dickenson & Dann (1932), "it is possible that a deficiency of some dietary constituent is the essential cause of haematuria," and from the result of urine analyses carried out by them, they add



that "there appears to be some possibility of a low protein intake and possibly a low sulphate intake." Besides this idea of a chemical or nutritional deficiency, there is, of course, the other suggestion of Law (1901) that "some special poison in the pasture may be the unknown cause of the disease."

(2) Soils.

Closely associated with vegetation is the question of the soil of haematuria farms, because the character of the latter would naturally be reflected to a large extent in the former. The character of soil conditions determining the incidence of certain animal diseases of specific or nutritional origin, like Johne's disease or osteomalacia of cattle respectively, or to put it in another way, the influence of the soil upon the welfare of the animal body through the intervention of the herbage, is being increasingly realised in animal pathology.

Regarding haematuria, land reclamations (Pichon), thin arable soil poor in phosphoric acid, poor feeding (Cruzel) were suggested as associated factors about the middle of last century. Landel and Stricker ascribed the disease to soil conditions, peat soil in particular. The leaching of open soils by excessive rains has been mentioned by several authorities as being present in the affected farms.

In Belgium, most of the cultivators believe that the deficiency of lime and phosphoric acid content of the soil in the Ardennes is the cause. After prolonged investigations, Schlegel states that the disease is related to calcium deficiency in the soil, produced by the washing away of this constituent through heavy rains. These soils are stated to be rich in potash and silica content. Similarly, Gottardi (1935) in Italy ascribes the disease to soil conditions producing deficiency in calcium and phosphorus in the blood of affected cases. In Canada, the probability of a nutritional disturbance and of a mineral deficiency, hyperacidity, or an unknown factor in the soil and vegetation, has been suggested (Ottawa Report, 1930). Hill, King & Laird's (1933) analytic data relative to haematuria farm soils, show that the total insoluble matter, representing chiefly insoluble silicates and certain forms of organic matter, is comparatively high, also that the soluble silica in the form of soluble silicates is slightly higher in some instances than normally expected. Further, the statement by them that the lime content is not abnormally low, and its relation to magnesium content is within a safe range, is against the results of Schlegel.

The Canadian workers have made the further interesting, and it will be shown later, a possibly

significant observation, that the hydrogen ion concentration of the affected soils is acidic, and the ratios between the calcium, iron and aluminium would suggest that the cause of the acidity of these soils is probably the existence of iron and aluminium compounds which are held absorbed by the soil colloids and associated with the silicates. Analyses showed that the soluble silica, aluminium and iron are comparatively higher on affected farms and their range is wider. Recent Australian workers assert that there is no appreciable difference between the soils of haematuria farms and non-haematuria farms. Yet they quote Prescott and Piper to show that in haematuria areas "the soil is remarkable for its high fertility in terms of plant nutrients and its favourable fine sandy, loamy texture, tending to a degree of looseness, locally expressed by the term snuffiness." They visualise the possibility that certain soil conditions lead to some abnormality in the herbage which is directly responsible for the production of the disease. In dilating on this point, they add that this abnormality may be due to the absence of some rare element or organic substance, or may be caused by the presence of an abnormal organic substance originating from an abnormal metabolism of the plant.

(3) Altitude.

It has been stated earlier that the habitats of the disease are provided by mountains or sub-mountainous regions, and in some cases by bench lands which may be only a few feet above the sea level. Some actual data exist in this connection, and these would appear to be worthy of note.

In Bulgaria, haematuria farms are situated on high-lying lands at an elevation of 2500 ft. to 7000 ft. In Germany, the enzootic areas lie at a height of between 550-600 metres. In France, the disease is said to occur mainly in low-lying areas and scarcely above 2400 ft. In Yugoslavia, which appears to be the only country where the disease is present in every county though to varying extents, one finds that the lowest area, the Banat of Danube, has the least percentage of land area affected, while the Vrbas Banat has the highest. In Jamaica, the disease occurs in the mountainous regions of Southern Manchester, while in the Pacific Islands it is seen at an elevation of 3000 ft. to 4000 ft. In the Republic of Colombia, the affected farms lie at an altitude of 1000 to 3000 metres. In British Columbia, the affected farms may be as low as 40 ft. to 350 ft. above sea level.



A consideration of the above cited specific data would seem to demarcate the maximum and minimum ranges which are capable of supporting, or producing, the conditions required for the existence of the disease. The fact that the disease may occur at an elevation of 40 ft. only in one country, as opposed to its occurrence at 7000 ft. in another, can perhaps be explained on the relative elevation of the haematuria farm or district in relation to its immediate vicinity. The invariable association of elevated lands with the incidence of the disease has been observed in all countries, but the special conditions of Yugoslavia make the relation even more obvious. The significance of this would seem to lie in the amount of moisture conditions available, depending, of course, upon the rainfall, humidity, drainage, and the degree of diminished evaporation peculiar to wooded areas. Besides, the nature of the vegetations and the cultural conditions provided by the geological and surface soil formations, would also appear to have some important bearing. The relative part played by each of the factors in constituting a natural enzootic area, may only be discussed at a later stage. The statement of Fleming, Fowler & Clark (1930), that it is open to doubt whether haematuria occurs in places where potassium iodide

is being administered for preventing goitre peculiar to high altitudes, is of unusual interest. Excessive rains may lead to serious leaching of elevated lands, but goitre and haematuria are not found associated together. While iodine deficiency by itself is not expected to set up haematuria, any diminished incidence or suppression of the disease, as a result of iodine supplements, would require to be thoroughly investigated.

(4) Climate and Season.

Lienaux (1919) points out that one cannot say in which part of the year the disease starts but the symptom of haematuria is often exhibited first in the winter. Atmospheric influences seemed to be without effect, since he observed that the exceptional dryness of 1911 did not reduce its incidence from that usually seen in any moist or damp year. Bull, Dickenson & Dann (1932) remark that opinions regarding the disease being more common in one part of the year than in another, are quite unreliable. The annual rainfall in their area is said to be between 30-35 inches. Moussu states that in France the disease is often observed in the winter when the animals are housed, as well as in the other seasons when the cattle are at pasture. In Germany, more cases are stated to occur during and after

a dry summer. In Canada, the disease is seen more frequently at the end of winter. Recent Canadian workers state that the disease requires a long cool season, colder habitats and higher altitudes. The haematuria area of Canada has a mild winter and a cool summer, with a yearly average rainfall of 53 inches, a mean monthly temperature of 49°F., and with 1627 hours of bright sunshine in a year. According to the workers there, the two important climatic factors which are undoubtedly involved in producing the soil conditions found in the affected farms, are rainfall and temperature. Hill, King & Laird (1933) suggest that these may be the significant factors of the disease. They are convinced that the amount of rainfall must be the most important of all the factors in producing the changes in the composition of plants, which their analyses revealed. The occurrence of heavy rains in the affected areas in Germany, has been emphasised by Schlegel (1934).

(5) Water Supply and Drainage.

In Germany, the water supply in the affected areas is said to be poor in mineral salts. The spring water from notorious localities was tested experimentally on healthy animals, and from the negative results obtained, it was concluded that



the water supply of enzootic areas did not contain any virus or bacteria which might produce the disease. Further, the statement from Germany that one can distinguish the water of these localities on account of its high percentage of nitric acid content, is interesting. In Canada, Hill, King & Laird found that the water supply in none of the farms was highly mineralised or contained any elements in such quantities as might be detrimental to cattle. They analysed the silica content of <sup>the</sup> water supply (average 8.6 as  $\text{Si.O}_2$ , the maximum 12.4 to 13.4) and considered the data suggestive, when taken in conjunction with that of the herbage produced on affected farms. They refer to the extensive metabolism of silica, which takes place in milch cattle, and to the retention of this element from rations being surprisingly large. While considerable amounts of this element were found in the urine by them, its presence in milk was not in weighable amounts.

In Canada, the water supply of the affected farms is obtained largely from seepage wells, 12 to 30 ft. deep, with some surface water and artesian well water (Mackenzie, 1930; Grauer, 1930). It is understood that the pastures there are more or less badly drained, and that as a result of heavy rain-falls and the impervious nature of the sub-soil in

spots, considerable surface water accumulates which may stand for the greater part of the year. Cattle have access to the surface water in pasture fields or bush lands. Bankier (1936) asserts that the maintenance of cattle under such pasture or watering conditions, is the only apparently common feature among the affected farms, and this is also the most striking difference between the affected and non-affected areas. Further, it is noteworthy that the relation of the surface water to the incidence of the disease receives added support from the observation in Canada that the affected cattle recovered in many cases after the drinking water had been changed from the surface water, previously supplied, to artesian well water, (Jarvis), as well as from the other observation that if not too seriously affected, some cattle make recoveries on being removed to disease-free areas. (Lienaux, 1919; Grauer, 1930). When a comparative chemical analysis of surface and artesian well water was made, the most noteworthy difference revealed was the increased amount of salts (chloride, carbonate and sulphate of sodium, and bicarbonate of calcium), and the high iodine content (2000 parts in 100 billion) of the artesian well water.



(6) Geology of Haematuria Areas.

Hill, King & Laird (1933) state that geologically the affected sections of the Fraser Valley area are composed principally of glacial drift, overlain in places by small thicknesses of marine deposits which were laid down during glacial retreats of the pleistocene time. According to Johnston, quoted by them, the glacial drift soils occur on the uplands which were formerly, and still are, in large part, heavily timbered. They are mostly sandy or sandy loam soils. The upland areas are being gradually brought under cultivation in places where the valuable part of the forest has been removed in timber operations. The topography of the land is usually of a rolling nature, with occasional fairly level areas. The type of soil may be classed generally as coarse, sandy loam, varying from a light brown to a dark chocolate colour. It is usually acid in reaction. The texture is often open and readily leached, and drained naturally.

In Australia, the bedrock of the area consists of a series of marine tertiary limestones which extend over thousands of square miles to the north, west and east of Mount Gambier. The limestones include red and cream-coloured dolomites and a polyzoal limestone, and in places flints are abundant. The



limestones decompose to a red clay, and the chief modifying feature of the surface soils is the peaty accumulations of the extensive swamp areas, and the sand of the ancient dune ridges. The most fertile areas are those where basaltic "ash" fragments have been deposited. Most of the haematuria farms are found within the area of ash deposit in Mount Gambier. The soil is much less acid than most other South Australian soils. Some of the soils are unusually well supplied with plant foods, the maximum amounts observed being 0.4% of nitrogen, 0.4% of  $P_2O_5$ , and 1.7% of  $K_2O$ .

Regarding the geology of haematuria farms in Germany, the affected farms contain granite, gneiss and coloured sandstone.

It may be remarked here that a consideration of the available geological data from the three countries given above, does not seem to justify any clear generalisation being made.

Chronic bovine haematuria has received attention from many investigators. It occurs in many countries and has been known for the past century. The literature on the subject is, therefore, very extensive, widely spread, and written in several languages, some of which are not intelligible to any average English-speaking student. In recent times articles on haematuria have been fairly frequent, and several Theses on the subject have been presented to Universities in Europe and America. Yet no attempt has been made to put either the old or the new literature together. In fact, a lack of co-ordination among the various writers on the subject, seems to be a feature of the literature extant upon the disease. Investigations have been carried out along various lines, and though some quite lengthy articles have been written, records of definite data of significance are very few indeed. The failure to produce any significant data, in spite of prolonged investigations, has been commented upon from time to time by the investigators themselves.

Considerable time and labour have been devoted to the perusal of the existing articles. Without attempting a complete and detailed chronological account of all the literature, it is proposed to provide a bird's eye view, as it were, of the more

important and noteworthy contributions, particularly those that are likely to be of value in elucidation, or in filling up the gaps in our knowledge. A number of Theses and foreign and old literature could not be secured for reference.

Writing in 1787 about the evacuation of blood in the urine, Topham says: "For I am well convinced by experience during a course of sixty years' practice, that a sudden change of weather, seasons or climate is the most common and effectual cause of, not only this, but of almost all inflammatory diseases whatever." It is not clear if the writer implied an inflammatory process as the cause of haematuria, but, as the first reference, it is certainly of some interest. Anderson (1842) found in the urinary bladder of a cow a "fungus haematodes" in the form of five pedunculated blood sponges (fungus haematodes) which stood out of the vesical mucous membrane and "perspired" blood. Hubner, in the same year, observed an epizootic of haematuria of cattle at Meissen. He believed it to be due to Arnica, and buds and shoots of Coniferae, which cause an occasional haemorrhage and tearing of the vascular tissue. In an ox passing blood-stained urine, Vaes (1843) observed rupture of the urinary bladder and spongy growths on the thickened mucous membrane of the organ. The growths



were differentiated from the surrounding mucous membrane due to their greyish brown colour. Raconnat (1847) described cauliflower-like polypus in the bladder of a bovine as the cause of haematuria. Working in the province of Maine, Pichon (1863) ascribed the disease to changes in the cultivation, which had altered the general appearance of the country and the conditions of cattle breeding. The specific factors mentioned were land reclamations, the lime dressings applied to land, and the introduction of the Durham breed of cattle. In 1864, Sinoir considered that the crossing of the local cattle with the Durham breed had undermined their bodily resistance, and had increased precocity, making them highly predisposed to attacks of haematuria.

Both Pichon and Sinoir described changes in the kidneys and ureters, but did not attach any significance to these. They were inclined to consider haematuria as a blood disease, resulting from poor feeding. Reynal endorses this view and says that as a result of increased fluidity of the blood, the blood corpuscles become smaller than normal and can therefore escape through the wall of the blood vessels more readily. Reynal affirms that plethora causes haematuria, since under the prolonged

influence of a very assimilable diet, the blood becomes more plastic, circulates with difficulty in the capillaries, and may even rupture them, with a resulting capillary renal haemorrhage and haematuria. In Reynal's experience, the disease occurred when the cattle were turned out to the very rich pastures characteristic of the spring, and, similarly, he found the unusually rich pastures of Normandy notorious for producing bloody urine. Roll and Lousienne encountered an epizootic of haematuria in the Austrian Alps and in the region of Aupal, but no vesical growths were found, and the cause was not determined. Landel and Stricker observed haematuria in England and Holland, and gave the causes as soil conditions (peat soil), certain plants of Ranunculaceae and Euphorbiaceae, and water containing lead. Ross, in 1878, described haematuria locality as containing abundant hay and straw of poor quality, with deficiency of green grass, and with pastures containing standing water. Pflug (1876) thought that the polypi were caused by constant irritation in the chronic cystitis, and bladder calculi could form anywhere on the bladder mucosa. Galtier and Boudeau (1886) concluded that haematuria was a local disease of the bladder, due to a toxic irritant substance and secondary microbes which were excreted through

the urine. The organisms gaining a foothold in the bladder could produce great irritation, but when injected caused no haematuria. Cystitis haemorrhagica became necrotic and incrustaceous, and was a disease transmitted through fodder spoilt by "melioration" of the meadow. Semmer found bleeding was due to polypoid new growths in the bladder. Hink (1886) observed Stallrot (stable red), and Weidrot (pasture red) in cattle in the Black Forest. He found the latter in young stock in the early part of the year, ascribes it to the cooling of the body surface in general and of the digestive tract. In the peculiar and chronic disease "Stallrot", blood comes out of the bladder. Hink called it haemorrhagic inflammation, caused by obstructions in the way of the posterior vena cava, due to growths impinging upon it. Thus varicosity and engorgement of the capillary and venous blood system take place, eventually causing them to give way and form ulcers. Stockfleth, in 1889, writes of a neoplasm of the bladder of a cow. The bladder had an uneven surface, and the papillae and mucous membrane had the appearance of a carcinoma. It simulated a polypoid fibroma, but was really a scirrhous carcinoma. In 1890 Arnold published observations on haemorrhagic verrucose growths of the urinary bladder, apparently caused

by "gregarines" (coccidia) developing in the epithelium of the bladder mucous membrane. Hink and Lydtin had already considered this possibility.

Detroye (1891 and 1904) ascribed the disease to a microbial infection and considered it to be a readily transmissible condition. He thought that the specific cause was a micrococcus which appears in culture on serum, urine and bouillon as a diplococcus or a streptococcus. The organism resists desiccation for months. According to him, the micrococcus grew naturally in the moisture of the pastures, and the infection was contracted through feed or water. Young growing animals are more susceptible to infection with the bacterium than older animals. Of the seventeen transmission experiments, ten were positive. Treatment is useless. Clearing of pastures and isolation of the sick animals are the measures of prophylaxis recommended.

Galtier (1892-3) ascribes chronic haemorrhagic cystitis to the ingestion of irritant plants by animals which are already parasitised by the liver flukes (distomiasis). When thus parasitised, the liver can no longer perform its functions and destroy toxins effectively. The toxic principles of Ranunculaceae, sedges, rushes, etc., are absorbed and, without being previously destroyed, are eliminated



through the kidneys. The toxic principles, having passed to the bladder, cause intense irritation and haemorrhagic cystitis, which is maintained subsequently by microbic organisms present in the bladder. Cruzel suggested that the disease was entirely due to poor feeding.

Against Cruzel's view, Moussu (1905), who has done much to remove misconceptions regarding the disease, points out that the theory of poor feeding and poor forage does not explain the causation of the disease. Debilitated animals, for instance, pass through all stages of wasting and profound cachexia, but never pass blood in the urine, while haematuria is exhibited quite frequently in animals of good bodily condition. In controverting Galtier's ingenious theory, Moussu recounts the two simple facts, that haematuria is known to occur among animals with no liver fluke infestation in them, and, secondly, that the disease does not occur in the lower regions of the "departments" of the Nord, the Pas de Calais and the Somme, where both of the factors incriminated by Galtier, viz. Ranunculaceae, and other irritant plants, and liver flukes, are common. Similarly, Detroye's micrococcal theory falls to the ground, since the culture he sent to Nocard was found to be incapable of setting up

disease. Boudeau (1893) supported Cruzel's view by stating that haematuria affected as many as one tenth of the cattle population in the south of the Canton of Indre and the north of the Creuse, parts of which are characterised by thin arable soil, deficient in phosphates. He went further and suggested that the dressing of land with lime and phosphates in the affected areas would prevent the occurrence of the disease, as, in his experience, the disease had disappeared under such conditions from previously notorious farms.

Gmelin (1897) divided blood-stained urine into Weidrot (pasture red), as an acute haemoglobinuria, and Stallrot (stable red), as chronic haematuria, following upon a productive cystitis associated with papillomata and bleeding neoplasms of the bladder.

Lienaux (1905) discountenances all the existing theories regarding causation and seeks the cause in the abdominal circulation of affected animals. He considers that inflammations of the bladder wall result from the abnormal size of some of the organs of the posterior part of the body, and sometimes due to the defects in the lung function. In a later paper, Lienaux (1919) modifies his previous view and says that the disease is not seen in voracious eaters or as a complication of fattened animals, as

would be expected to happen if his previous hypothesis were correct. He criticises Detroye and says that the latter did not distinguish between haematuria and haemoglobinuria.

In extension of Lienaux's previous hypothesis, Delcroix (1905) incriminates pressure stasis of the venous blood, particularly of the pelvic veins. Moussu (1905) discusses the existing theories of causation but avoids making any definite suggestions himself, though when dealing with the subject of treatment, he makes the abrupt assertion that "the disease is beyond question of a parasitic character."

Goetz (1906) studied the pathology of "warty cystitis" in Switzerland. He described the presence of "water-clear, oval or round structures in the epithelial layer (of the bladder) with a granular content arranged in the form of clumps or rounded masses, and showing a more or less large nucleus-like body." He was definite that these peculiar bodies were different from the cells of the surrounding tissue. The size and shape of the bodies gave him the impression of "Coccidium oviforme," and reminded him of the previous findings by Arnold and others, of similar structures. He was inclined to think that they were secretory products having a "drop-like" structure.

Case (1911) examined sections of bladder growths from haematuria cattle in the Hawaii Islands, and makes the statement: "the study of the peculiar cells found in such large numbers in the (bladder) submucosa leads to the conclusion that they are the etiological factor in this disease." The cells are described as circular in outline with a central nucleus, rich in chromatin and taking a deep haematoxylin stain. In another form, the parasite exists as free nuclei. The nuclear forms become encysted in the epithelial cells, and he thinks the parasite should be classed as a Coccidium.

The above observation is certainly interesting, but curiously this has never received any notice in literature.

In 1917, Hadwen complained that in Europe apparently very little work was being done upon this important disease of cattle. By his experiments, he tried to prove that injections of dilute oxalic acid solutions provoke great irritation, and a bladder disease indistinguishable from bovine haematuria. He postulated that calcium oxalate crystals are formed in the bladder (from the ingested fodder, suspected to be oxalic-acid-bearing), as soon as the acid comes in contact with urine and mucus. The crystals, being characterised by cutting edges,



have a direct effect on the bladder wall, and it is probable that oxalic acid itself has a selective deleterious effect. After a time, contaminative bacteria play their part in aggravating and maintaining the lesions produced by oxalate crystals and oxalic acid. The blood drawn from one of the oxalic acid treated heifers, coagulated in thirty-three minutes. Andersen (1918) and Cleemann (1918) reported on cases of "parturient" haematuria. While making microscopic examination of bladder growths, Roger (1912) found that the branching growths were composed of very large ovoid cells, with a large nucleus disposed in several layers round a peduncle in the manner of petals of a flower. In the epithelial layer of the bladder, he discovered the presence of transparent ovoid bodies without much structural differentiation. These did not resemble any known parasite, and he believed they were degenerated epithelial cells. In four cases of haematuria examined, Cleland (1911) found numerous larvae of Pentastoma denticulatum as infesting the mesenteric gland. He did not find any evidence of the presence of actula larvae in the lesions or in any part of the bladder. He expresses the opinion that the disease is not of bacterial origin, since cultural examination and smear examination of organs have been

negative. Roger (1916) remarks that oxalaemia should have a place in veterinary pathology, and believes that this can play an important part in horses, as well as in cattle. In 1922, Ichikawa in Japan found adult Schistosoma japonicum worms in the bladder tumours of two bovines. In five other cases, he failed to detect any bilharzial worms. Mathis and Leblanc are said to have incriminated Schistosomes in 1897 and 1893 respectively. Craig and Kehoe (1923-25) stated that the causal agent is probably some chemical irritant in the grass or fodder from the land, which is excreted in the urine, but they are unable to identify any special plants with the production of the disease. They fail<sup>-ed</sup> to find any chemical agent to account for the condition. While discussing the various theories, Lahaye and Rulot (1926) question Hadwen, and express the opinion that the disease is caused by an intoxication produced by a species of Mercurialis. In the experiments carried out by Kalkus and Sawyer, oxalic acid and oxalates failed to produce haematuria. Miyamoto (1927) studied the disease in Formosa among cattle and buffaloes. He portrays the development of various types of neoplastic formation in regular sequence. He reports that cystitis and pyelonephritis occur due to secondary infections, and numerous

streptococci in long chains may be present. Cocheril (1930) ascribes the disease to intoxication due to Mercurialis, similar to the view of Lahaye and Rulot noticed above. Allardyce, Fleming, Fowler and Clark (1930) were unable to detect any notable difference in the constituents of the blood of haematuria and normal cases. Cholesterol, sugar, non-protein nitrogen, urea nitrogen, creatine or creatinine, calcium, inorganic phosphates and chlorides were estimated. In Fleming, Fowler and Clark's experience (1930), two remedial measures were found to be beneficial: (1) A change of drinking water, from surface to artesian well water, (2) the administration of ground coral rock. Scharer (1930) says that the disease is not spontaneously cured, and describes the finding of "coccidia" in various developmental stages in the tissue cells of the tumour-like growths of haematuria. Durin and Unglas (1931) believe that the disease is a form of Colibacillosis, and attempt treatment in that belief. Bull, Dickenson and Dann (1932) state that a bacteriological examination of the bladder lesions in earlier cases has failed to reveal the presence of any micro-organisms. They add that one or more small and insignificant lesions may lead to severe, and even fatal haemorrhages, while large or more extensive

lesions may bleed very little. Further, in their experience, the commonest lesions found apart from the urinary system, are haemangiomas of the liver, hydatid cysts in the liver and lungs, pentastomiasis, and occasionally oesophagostomiasis. Lockett (1932) reports that in Jamaica mildly affected cases have been observed to recover permanently on a more or less complete change of food and water conditions. He failed to find any gross parasites in affected animals. He obtained temporary improvements with intravenous injections of tartar emetic. Mackenzie (1932) carried out an economic survey of the disease in Canada. Giovine (1933) ascribes the disease to the condition of the fodder, and states that the changing of the pasture reduces the incidence. Hill, King and Laird (1933) analysed soils, grass, and oat hays, and samples of water from haematuria farms, and also similar materials from definitely disease-free areas. Simonetti (1934) ascribes haematuria to Babesia bovis.

Commencing with Ross's work in 1878, Schlegel (1934) surveys the literature. The previous forty years' literature existing on the subject would appear to be unknown to the author. Schlegel wrote his first article in 1912. With the approval of the Reich authorities, a very comprehensive investigation



was started in 1927, and all the work to date is recorded in an exhaustive series of documented articles. In collaboration with a geologist, a botanist and a chemist, he carried out a complete scientific survey of the haematuria localities, the so-called "hunger farms" of the Black Forest in Germany. Details of the geological and chemical nature of the soil, the water supply and botanical flora of the farms were studied. The chemical and physiological characters of the blood and urine, the clinical and post-mortem features associated with the disease, have been studied. Records of the observations upon the various aspects have been interpreted in the light of the existing knowledge upon the mineral metabolism, role of vitamins, blood constituents, the physiological mechanism of the clotting of blood in normal cattle and its retardation. The author attempts an explanation of the causation on these lines, and believes that a haematogenous toxin, derived from local plants, is the cause.

Gottardi (1935) studied chronic haematuria in milch cattle, and states that the water of affected farms was rich in acids, due to soil being deficient in lime. He ascribed the disease to a diminution in the calcium and phosphorous content of the blood.

Bankier's (1936) investigations consist chiefly of field observations regarding the conditions under

which the disease occurs, as well as of cattle feeding experiments on a notorious haematuria farm at Milner, B.C., which was specially leased for investigational purposes. Attempts were made to determine if the forms of insect life present in the surface water, had any relation to the disease. Bankier reports the finding of quite large cells in urine samples, but is uncertain about their identity and significance. He goes on to state that, of the reports of scientific workers on the disease in other parts of the world, the most interesting one appears to be by Datta, who claims that the disease in India is caused by a parasite. No definite proof has, however, been secured yet of the disease in British Columbia being caused by such a parasite.

Pommeret (1937) records the results of his studies upon samples of haematuria urine and bladder, collected at the abattoir at La Villete. He states that on examination of the urine of twenty cows and of twelve specimens of diseased bladders, he succeeded in two cases in discovering the bodies described by Datta as *Entamoeba*. He asks the question: "Le bacille de Pasteur et Thuillier donne le rouget au porc, le bacille de Nicolaier et Kitasato donne le tetanos, mais quel est l'agent de l'hématurie essentielle, nous l'ignorons. Cependant, n'y a-t-il pas lieu de

fonder quelque espoir sur la theorie toute recente qui nous vient des Indes sous la plume du Capitaine Datta?"

In Yugoslavia, Butozan (1938) has worked out the percentage land area in each county and circle which is affected. He describes how the disease exists to varying extents in all the counties there (Banats), the least affected being the Banat of Danube (1.9 per cent), and the most widely affected being Vrbas Banat (68-76 per cent). He has carried out chemical estimations of the inorganic constituents of the blood and urine, and has found no parasites in the blood smears from affected cattle. He records results of the observation for one complete year upon two cows and one bull.

In summarising the foregoing narrative, it may be recalled that no definite clues or indications have yet been given by the literature extant upon the disease, and the theories that have been propounded have been declared to be unacceptable by various workers (Hadwen, Kalkus, Grauer, Schlegel). Nevertheless, an analysis or examination of the basis of the theories so far put forward, may be worth our while.

Generally speaking, all the foregoing theories are based upon some individual observation or other relating to

(1) the tissue changes seen in affected animals (e.g.

due to "fungus haematodes", Anderson; cauliflower-like polypus, Raconnat; chronic cystitis aggravated by constant irritation, Pflug; due to polypoid neoplasms, Semmer; round cell sarcoma, Hink; Scirrhus Carcinoma, Stockfleth; transitional neoplastic growths, Miyamoto; mechanical passive hyperaemia, internal sarcoids, pressure stasis);

(ii) microscopical findings of organised factors, bacteria and entozoa, in the diseased tissues (e.g. due to micrococci, Detroye; secondary bacteria, Galtier and Boudeau; colibacilli, Durin and Unglas; due to filaria, distomes, Lydtin, Hink; schistosomes, Leblanc, Mathis, Ichikawa; and pentastomes, Cleland; due to coccidia, Arnold, Goetz, Case, Roger and Scharer);

and (iii) clinical observations on environment (e.g. due to the influence of the local vegetation, Pichon, Sinoir; soil and pasture conditions, Ross, Galtier, Boudeau; lack of cultivation and lime dressings, plant poisons, mineral deficiency, oxalic-acid-bearing plants, chemical irritant in the grass or fodder, Craig and Kehoe; haematogenous toxin derived from local plants, Schlegel; toxic irritant substance through fodder spoilt by "melioration" of the meadow, Galtier and Boudeau; faulty metabolism, amelioration or cure due to change of place and elimination of surface water).



Regarding the investigations which are now in progress in different countries, it may be noted that they are being approached from one of the two angles, viz. (i) the phenomenon of enzooticity -- what inter-relations of factors govern the incidence of the disease -- and (ii) direct observation of factors operating in the lesions in animal tissues. Of these two angles, the former has been receiving a greater amount of attention, but while no single environmental factor or specific plant or poison has been identified with the disease, it is generally agreed that the environment does play an important part. The observation that the disease is associated with farms where much of the land is weedy and out of cultivation, and that the disease disappears, or is reduced in incidence, after agricultural improvement, has been confirmed from most countries (France, Kenya, Columbia, New Zealand).

Turning to the organised factors said to have been seen in the affected tissues, it is necessary to remark that the association of any readily recognisable gross parasites is out of the question (Craig and Kehoe, Lockett, Schlegel). Bodies somewhat similar to coccidia have been repeatedly seen (in Germany, Switzerland, Hawaii Islands, France, Antiquoa). While it is certain that the disease is

not a form of coccidiosis, the findings of coccidia-like bodies have not yet been satisfactorily explained or convincingly interpreted. Is it possible that these bodies represent the essential factor in the weedy environmental conditions, the specific poison, irritant or haematogenous toxin peculiar to the local vegetation, the oxalic-acid-bearing plant from which the crystals of oxalates are introduced into the urinary bladder? The deficiency of green grass, the abundance of hay and straw of poor quality, acid conditions of the soil, are said to characterise the affected farms. Is it possible that the coccidia-like bodies are favoured by the above conditions? The disease is exhibited only after animals have lived and grazed in special pastures, yards and stables for prolonged periods. Does the time factor help the coccidia-like bodies to increase in number and reach a density of growth bearing a significant relation to the incidence of the disease?

## SECTION II.

THE PRESENT INVESTIGATION.I. Introduction.

The writer has been engaged in the study of a variety of problems relating to animal diseases\* and animal husbandry in India for over twelve years. In the course of his technical duties as veterinary pathologist at the Imperial Veterinary Research Institute, the only central institute catering for the needs of the Indian sub-continent, he has had unusually good opportunities for studying at first hand a unique array of obscure diseases as they occurred throughout the length and breadth of the country. Local investigations were made in the field, and interesting materials brought up to the laboratory headquarters on the Himalayas (Mukteswar), and subjected to thorough detailed examinations and animal tests, such as would not be practicable under conditions obtaining in the field. During the period of his service, the writer has had to make, as a routine measure, thousands of autopsies upon both large and small animals, and to examine hundreds of histopathological sections and smears from each interesting animal, representing one or

\*Writer's results incorporated in NeveuLamaire's (1936) Helminthologie, Monnig's (1934) Helminthology, Cameron's (1934) Internal Parasites, Curasson's (1936) Pathologie exotique, Hutyra & Marek's (1938) Special Pathology etc..

other of the diverse disease problems peculiar to that country. Thus research work has progressed by degrees, though be it through vicissitudes and pitfalls. Months and years of strenuous efforts have rolled by, recording in some few cases definite advances leading up to publications, while in the larger number of cases only negative results have been obtained, requiring further and further concentrated thought and experimentation. The faint glimmer of light now and again brought to bear upon the problems, by the creation of a working hypothesis, has at times altogether disappeared, leaving no other alternative than mere groping in the dark. Although the above remarks, made in a general way, represent the early experiences of the writer in connection with some of his previous original work, it may be mentioned that in no other field of research has he been more mystified, stage after stage, than in the case of the investigations upon chronic bovine haematuria.

While posted at Calcutta in the early part of the writer's service, the late Lt. Col. H.W. Acton, I.M.S., then Director of the Calcutta School of Tropical Medicine, was himself actively pursuing problems of medical mycology, as applied to the Tropics. In recognition of that valuable work,



Dodge has created the genus Actonia. Acton was kind enough to initiate the writer into the mycological methods that were being employed in human pathology, and pointed out that if such methods were applied to the domain of animal pathology in India, interesting results might emerge. The suggestion was borne in mind, and a number of well-known mycotic diseases of animals, trichophytosis, actinomycosis, Cryptococcus farciminosus infection, etc., were studied along with a number of obscure diseases, like bursati and lichen tropicus in horses, and bovine nasal granuloma. Having been thus drawn into mycological problems, the writer discovered in 1929 a new fungus, Eidamella actoni, as producing a peculiar form of dermato-mycosis in the dog (Datta, 1932), and then started the investigation upon bursati in horses, where Holmes (1914) had incriminated a Sporotricum sp. as the causative agent, and later upon nasal granuloma in cattle, where Krishnamurti (1922) had incriminated a streptothrix organism. The etiology of both the diseases was thus discovered (Datta, 1933 and 1932 respectively), and subsequently confirmed by others. Thus the fungus theory in each of the above cases was finally overthrown. It may be interesting to comment here that the writer, (who has been

responsible for proving that certain diseases, which were thought to be of mycotic origin, were not of such nature), has himself been engaged in proving that the etiological agent of chronic bovine haematuria actually happens to be a mould-fungus, referable to the genus Aspergillus. It has taken the writer years of self-criticism and much cool deliberation to accept a mould-fungus as the cause of this particular animal disease, not because he was inclined to question the probability of such a thing, but because the addition of such a widespread disease of the urinary system in the list of the already known forms of Aspergillosis, was likely to have important bearings on the whole subject of Aspergillar infections. In the years that have been passed in these investigations, a progressive, though rather slow unfolding of the problem has taken place, and sufficient clear data have accumulated to make the conclusions irresistible.

The investigation upon bovine haematuria was commenced by the writer while in Bengal, and it has been continued at Mukteswar since his transfer there in 1930. Some of the work already done in India has been repeated at Edinburgh, to check up and consolidate the results. Some details of mycology, which were at first confusing and in the

interpretation of which the literature appeared to be ambiguous, have had to be restudied under the changed surroundings available at Edinburgh. The accessibility at this place of some of the relevant mycological and other treatises has been of considerable help. It is to be noted that the subject matter is primarily a problem in animal pathology. Just as in the writer's previous pathological researches, details of systematics (mycology, bacteriology, helminthology, etc.) were necessitated in some degree or other for the satisfactory solutions aimed at, the needs of the present researches have involved a considerable amount of purely mycological studies, to enable the proper understanding of some features underlying the biology of the organism in the animal system. With regard to the exact scheme and sequence of presentation which has been adopted in this thesis, it should, for purposes of clarity, be explained that the existing state of knowledge has been surveyed, and observations as they relate to the present investigation, (extending beyond the incidence of haematuria in India), have been reserved for the latter portion of this Thesis.

Chronic bovine haematuria would appear to be one of the most outstanding among diseases of domesticated animals. For, not only has it baffled

elucidation, notwithstanding the very considerable amount of scientific investigation which has been expended upon it, but what is more striking, workers even in recent times have been at a loss to find any interesting clue, worthy of being pursued with a reasonable prospect of success. The disease occurs in most parts of the world. While on the one hand, it is a comparatively widespread condition, on the other, it is peculiarly enzootic in its incidence. It is curious that while in the early stages the effects of the disease are so mild as to escape notice for a considerable time, yet once established, it is so intractable that it defies all the remedial measures that have so far been given a trial.

The continued failure of investigators to evolve effective methods of prevention and cure, must be largely due to the etiology being obscure. It is said that farmers in the Darjeeling district in Bengal dread this disease more than Rinderpest.

The inherent difficulties of investigating this problem have no doubt militated against its straightforward elucidation, but the evidence in literature of the remarkable persistence in attacking the problem shown by some of its investigators (Schlegel, Lienaux), who have not allowed their enthusiasm and keen interest to flag for as long as fifteen or



twenty-five years, is a sufficient testimony to the fact that at least the "doggedness" of some workers has been quite equal to the resistance of the problem to be surmounted. The history of scientific advances repeatedly shows that in the absence of technical methods (of examination, staining or culture) appropriate to each case, pathogens may at times be as highly elusive as in this case. Bovine haematuria has formed a subject of research for the past one hundred years.

It is not possible to ascertain when and where this disease first made its appearance. In the past, <sup>red</sup>all/discolorations of the urine, irrespective of their nature, whether or not associated with any local or systemic disturbance, have figured together in the language of the ordinary lay farmer, or even of the early veterinary surgeon, as "Red Water". In Great Britain, the use of the term has, for some time past, been restricted to a specific haemoglobinuria, that is due to the intracorpuscular protozoan parasite, Babesia bovis, which is transmitted through the agency of the cattle tick, Ixodes ricinus. It is unfortunate that the unscientific term "Red Water", as applied to haematuria, is still to be found in the latest veterinary literature from certain parts of the world. Apart from this unhappy

use of a vague term, the old state of what may be termed the confused lumping of chronic haematuria with other separate disease entities, has largely disappeared. A solitary delinquent may occasionally be found, but the present day veterinary periodicals contain sufficient evidence to prove that veterinarians everywhere have recognised it as a precise and well-differentiated entity, unmistakable for any other condition.

As is well-known, the term haematuria signifies the passage in the urine of the solid or formed constituents of blood (i.e. actual blood with unbroken red corpuscles). Leaving aside the discoloration of the urine, due to an admixture with the blood pigments liberated by the breaking down of red corpuscles, as occurs in haemoglobinuria, various types of pathological processes affecting the urinogenital system can lead to haematuria. The blood or blood corpuscles may become mixed with the urine, either in the kidney or in the urinary passages including the bladder, or be derived from the adjacent genital organs. In cattle, haematuria of renal origin is generally of the acute type, following upon trauma or acute infective diseases, like anthrax, purpura, or due to the rupture of the renal artery, or to inflammatory conditions, tumour formations, calculi,

and haemorrhagic diathesis, or due to infection. Haematuria, in the course of epizootics, has been recorded from South Africa in 1891, and from Russian Turkestan by Yakimoff (1926). The ingestion of several irritant plants, and of irritant diuretic drugs, is known to produce an acute haematuria. Further, there exists a special form connected with pregnancy and early parturition. Vesical growths may lead to haematuria, and according to Beer (1935), little exact knowledge exists on the origin of different types of bladder growths. Apart from the well-known incidence of bladder tumours among aniline dye workers in Germany, and sometimes in other countries, and the association of bilharzial cystitis with papillomata and malignant neoplasms, there appear to be several lesser known conditions affecting the urinary bladder. It is recognised that a pronounced epithelial proliferation can take place in cases of cystitis of longstanding. A few cases of amoebic cystitis, mycotic infections of the bladder, and occasional cases of bacterial cystitis, particularly due to B. coli and streptococci have been recorded in literature. From the University of Nevada, Records and Vawter (1928) have described a form of "red water disease" of cattle, which is said to be prevalent throughout Nevada, California,

Oregon, Washington, and Chili (South America). No details of the causative organism have been given, but it is stated that it is a bacillary haemoglobinuria for which a satisfactory curative serum has been produced. This record is of interest in connection with the differential diagnosis of haematuria in Washington and Oregon.



## II. THE AIMS AND SCOPE OF THE PRESENT INVESTIGATION.

Whenever a disease is encountered for the first time in a country, it becomes necessary to define its character, to study its relations, and to ascertain to what extent it conforms to any described condition from the same or any other country. It thus becomes incumbent upon the investigator to survey the ground that has already been covered, and to seek indications regarding the lines on which the proposed study may proceed. Such a procedure is helpful not only in controlling the work as it proceeds, but in checking up the results of past investigators, while guiding future work and guarding it from pitfalls and blind alleys.

Before outlining the aims and scope of the present investigation, it seems pertinent, therefore, to survey the extent and limitations of the past studies, as also to recapitulate the methods that have been employed. Although the study of the problem as it occurs in India should be developed on right lines, it does not follow that such techniques as have failed should be avoided. On the contrary, the application of the same methods of study in the different habitats of the disease, may bring out altogether new facts.

A survey of the past work shows that investigators have studied the disease in its clinical manifestation in the living subject, as well as by autopsical examination. Both the naked eye and microscopic examinations of the urine, blood and visceral organs and tissues have been made from time to time. Chemical analyses of the blood and urine have been repeatedly made. All the facts connected with the incidence of the disease, with particular reference to the age, sex, heredity and breed, have been analysed. The percentage of the infected to the total population, and any variations from season to season, or year to year, in a herd, has been recorded. Besides these factors, all the details of housing, watering, feeding and grazing, in fact all matters of general husbandry, have been scrutinised. The nature and source of the water supply has been closely examined for the presence of any abnormal or deleterious constituents. The nature and composition of the pasture and the neighbouring vegetation in affected farms and areas, have been compared with those from disease-free localities. The flora of the places has been subjected to repeated and detailed botanical and chemical studies. In regard to these studies, which have been carried out both intensively and extensively, the Australian workers

comment that the ordinary orthodox chemical analyses do not throw any definite light, and though they suspect the diet, they feel that a direct attack upon the problem by chemical analysis of the feed, would be impracticable without some indication of the nature of the substance to be searched for. Feeding tests with large quantities of suspicious plants have been carried out in both large and small animals. Besides these, numerous transmission experiments with various tissues or urine collected from diseased animals, have been attempted. Different commercial acids have also been tested by workers, because of their presence in the feed or the water.

While the above brief resumé roughly indicates how very comprehensive the past investigations have been, it also reveals the innate difficulties of scientific investigation in the absence of a working hypothesis, definitely based upon some substantial fact. That great master of veterinary research, Arnold Theiler (1929), when called upon to advise the Australian Government on bovine haematuria, expressed himself thus: "What is required is a Laboratory in Mount Gambier itself and preferably on one of the affected farms. The remarkable coincidence of phosphorus and manganese deficiency in this area should not be lost sight of. A clue to

further research may then be found." That the absence of a clue may so seriously militate against the effectiveness of scientific methods, admitted by such an experienced worker, is a further proof of the role played by the investigator's eyes and mind in recording and interpreting the constituent parts of a disease process or any other natural phenomenon.

The results which have accumulated from the various investigations, carried out by different workers at different times and in many countries, are certainly of value, though unfortunately no clear conclusions have yet emerged. The etiology of the disease still continues to remain obscure, and to produce economic losses and a feeling of uncertainty among the owners of the affected farms. Regarding the present trend of research, it is observed from the recent publications that most, if not all, of the work now in progress in several countries is concerned mainly with chemical methods being applied on environmental factors, besides a certain amount of field investigations, (including geology, meteorology, botany, chemistry of the soil, water and vegetation), in the belief that a chemical deficiency or imbalance is at the root of the trouble.

Having thus seen the extent and limitations of the past researches, as also the present trends, it



may at first sight appear unprofitable to attempt any further investigation, as all the available methods at the disposal of the investigator appear to have been fully tried, and more so, because the reputation and capability of some of the workers upon the disease have been of a high order. It is, however, not the tradition of Science to accept defeat and "give the game up as lost." It is no wonder then that teams of workers are engaged at present in some countries, while solitary investigators are none the less actively pursuing their studies in the field or in the laboratory. Supposing that a new suggestive, or maybe significant, finding were to be recorded by some of the investigators engaged upon the problem, it would mean that the finding, to be acceptable, must be capable of explaining all the known facts regarding the disease, including the failure of past investigations, notwithstanding the employment of recognised methods.

It now becomes necessary to state that in the writer's investigations the primary aim has been the same as that of his predecessors and contemporaries, namely to elucidate the obscure etiology and thereafter to evolve rational lines of cure and control of the disease. Special staff and establishment specifically for haematuria investigation have

been engaged in some countries, but the vastness of India's veterinary problems and the present paucity of its trained workers, have not allowed of any such idealistic scheme to mature there. While some of the writer's contemporaries have been fortunate in having the full collaboration of geologists, chemists and botanists in investigations upon this problem, the writer has been pursuing his studies from his own angle and alone. It is to be noted that the writer's researches are not confined to the disease as it occurs in India, but include morbid materials for comparative studies obtained through the courtesy of workers in Australia, Canada, Formosa and Ireland.

### III. THE DISEASE IN INDIA.

In India the term haematuria seems to have been employed for the first time by Kristnasamiengar as late as 1896. From the symptoms of the disease as seen by him among Mysore cattle, it appears evident that he was dealing with haemoglobinuria only. The existence of bovine haematuria has not yet been reported from the part of the country where he was working. The first true cases of haematuria were described from the Nilgiri hills by Rangaswamy (1922), and by Parameswara Ayyar (1922). Attention to the occurrence of the disease in Bengal, in the Darjeeling district, was drawn by Kerr in 1925 in official correspondence. The disease had already been encountered during routine post mortem examinations upon hill cattle at the Mukteswar Laboratory in the United Provinces. Specimens of diseased bladder showing the characteristic lesions of what was then thought to be a new disease, were collected and preserved for examination as and when time permitted. At a later date, information regarding the existence of the malady in the Kulu valley in the Punjab, became available. Further, the report from the last mentioned area added that haematuria had also been seen among human beings there. Haematuria

has since then been receiving some attention at different places in India, including the provincial laboratories, the Mukteswar Institute, and at the places of occurrence. Haematuria has figured in several Annual Reports of the Mukteswar Institute since 1925, and occasionally also in the provincial reports from Bengal and Madras. In the first case of haematuria in the Nilgiris, reported by Rangaswamy (1922), schistosome ova are stated to have been seen in the urine. In the samples of urine from two other cases of the disease from the same locality, Parameswara Ayyar (1922), however, records negative findings.

In point of sequence of time, the early investigation of the present writer, carried out at Calcutta, may be mentioned here.

The earliest cases that he was called upon to investigate were specially brought down to Calcutta from one of the worst affected areas in India, namely, the Darjeeling district. These cases were cross-bred animals in the advanced stage of the disease. They were obtained free for the investigation, as they were not of any economic value. These animals were three in number and were housed separately in horse stables. As the animals had come down to a hotter place from their cool mountainous home,



they were housed with as much comfort as the available facilities of the place would allow. Highly nutritious diet with special supplements was provided for them. They were kept under close and prolonged observation during the day. Temperature, pulse and respiration were noted, but nothing of interest emerged. The visible mucous membrane was examined, and blood smears from the ear vein made. Samples of urine were collected in clean bottles as and when the animals naturally urinated. All three cases were passing blood on arrival, and continued to do so till they finally succumbed within about three months of their arrival in the new surroundings. The blood smears were stained and examined but no parasites were detected. Similarly, the fluid urine or urinary sediments were examined in wet preparations and in stained dry smears, but excepting for types of epithelial cells, blood cells, and some coccal organisms which were single, paired or chained, nothing else was detected. Some of the chained forms were remarkably long.

As and when these animals died, post mortem examinations were carried out carefully and in great detail, but excepting the presence of numerous hydatid cysts in the lungs and liver, the only readily recognisable lesions were in the urinary organs.

The kidneys presented varying degrees of dilation of the pelvis and cyst formation in the parenchyma.

The urinary bladders showed large and small nodular elevations or warty growths on the mucosa. The inside of the bladder showed discolorations due to recent and old haemorrhages. The mucous membrane was mostly intact, but here and there a few surface breaches were present, some of which were just visible to the naked eye. On cutting into the organ, haemorrhagic areas were seen at different depths of the bladder wall. The thicknesses of the bladder wall varied to some extent in the three cases.

When microscopic sections were prepared and stained by various methods, the findings conformed to the features described from other countries. The investigation of the Calcutta cases terminated with the making of the histological examination of the tissues from these animals. While some idea regarding the disease was formed, nothing beyond the ordinary findings in these cases were recorded. The writer was then working at the Calcutta School of Tropical Medicine, and the preparations from these cases were made there, and were inspected by the late Colonel Acton, who was of the opinion that the condition resembled "internal sarcoids" or acquired angiomata of human medicine.

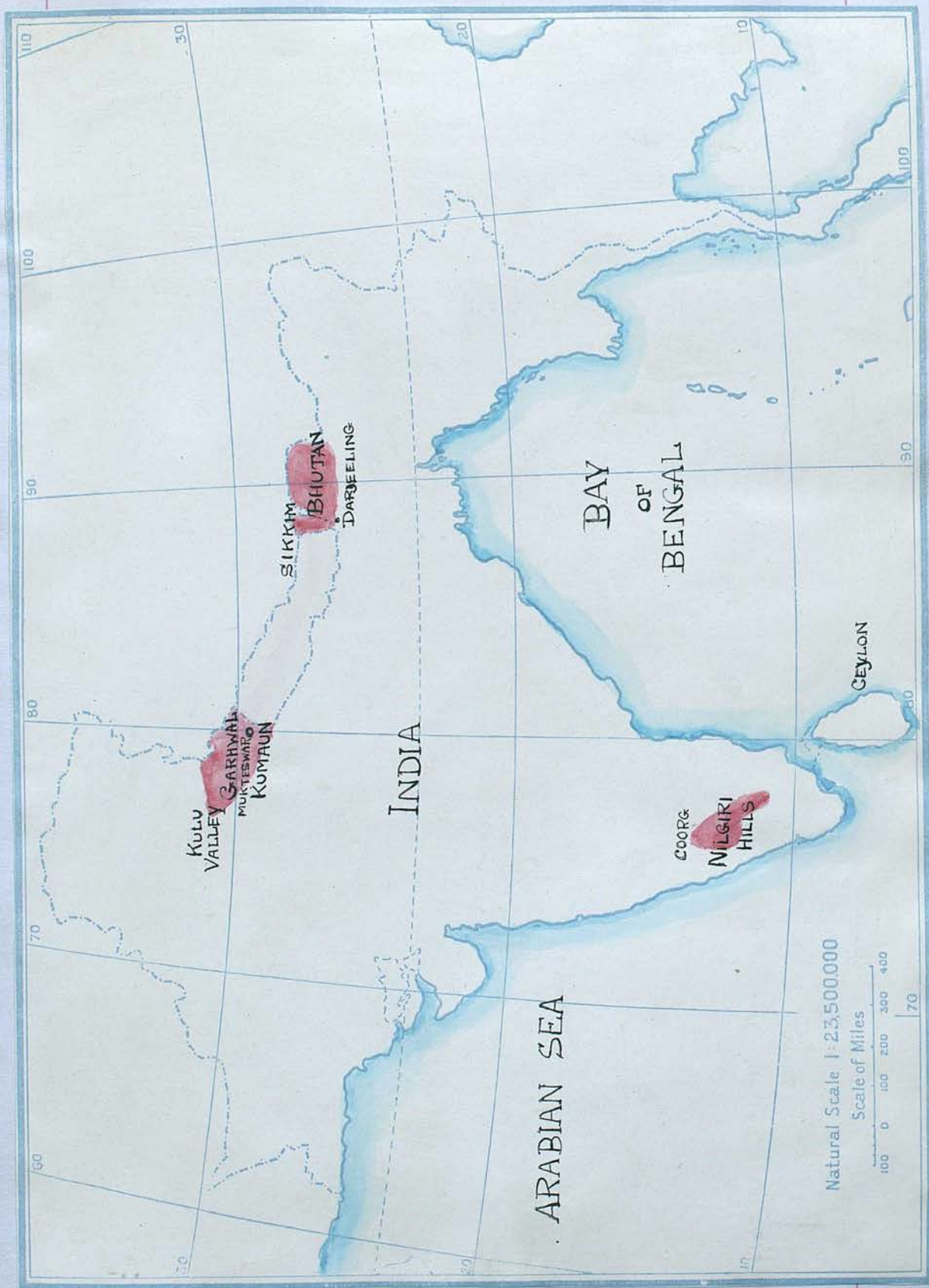
At this stage the writer was transferred to the Imperial Veterinary Research Institute at Mukteswar, where a few clinical cases of haematuria had already been collected, and specimens of diseased bladders were awaiting examination. The opportunity of continuing the studies already started in Calcutta was thus forthcoming, and the problem developed from stage to stage during the last few years. The existing literature on the disease was examined critically. In view of practical difficulties, Datta (1931) was not prepared to accept Hadwen's views, but considered that an endogenous production of oxalic acid and oxalates, due to disturbed metabolism, seemed a more satisfactory alternative. Subsequently, data of considerable value and promise were mentioned in the writer's Annual Report for 1931-2 (p.15). The observation of a definite host reaction against foreign or parasitic bodies with macrophage-like action, and resemblance to an Entamoeba was recorded in the Report of the following year (p.15). These bodies were tentatively thought to be a large protozoan parasite, with the power of ingestion, characteristic ring-shaped nucleus with a central dot, and vacuolated cytoplasm and occasional cytoplasmic prolongations (pseudopodia or buds). Stained sections of bladders, liver, kidney and duodenal growths revealed

similar parasitic bodies. The presence of these was confirmed in both wet and stained dry preparations of haematuria urine, but samples of healthy urine failed to reveal them. In view of the situation which developed and is referred to below, a preliminary report of the writer's investigations up to date was published in 1934.

In the Darjeeling district in Bengal the local people are said to dread the disease. They believe bamboo and fig leaves, which are common fodder there, to be the cause of the disease. Bamboo leaves (N.O. Gramineae; Bambusa arundinacea) are used by Indian villagers in cases of retention of the placenta in the cow apparently with a beneficial irritant action, simulating to a certain extent the effects of mild abortifacients, and Garudacher (1930) has reported the presence of hydrocyanic acid in bamboo leaf extract. Again, Mustak Hussain (1930) in the United Provinces incriminates "tinpattia grass", which presumably refers to an Oxalis sp. From the Calcutta School of Tropical Medicine, Ghose (1933) records the results of chemical analysis upon the leaves of plants suspected of causing haematuria, e.g. Schima wallichii, Ficus nemoralis and the Indian cherry, and since saponins were detected in the first two, he states that feeding experiments with



the leaves containing saponins are contemplated at the School. In the columns of a daily newspaper the claim was first made that "Macgregor and his assistants (1934) have been able to trace the cause of the disease to a protozoan parasite of the genus Coccidium which ordinarily attacks the bowel, producing intense anaemia and dysentery." The old coccidial theory of Arnold, propounded in 1890 in Germany, was thus revived, but soon a further claim was advanced in the same daily paper (dated 6th February, 1935) that "the disease was due to worms (Distomum-Flukes-Lanceolate variety), the eggs having been found in the urine and in the bladder tissues of the affected animals." The Government of Bengal, however, issued a communique (dated 7th of March, 1935) to the effect that the above report was misleading, and that further searching tests would be necessary before it could be stated with certainty that the origin of the disease had been finally settled. A subsequent communique (dated 10th of September, 1935) stated that Maplestone, the helminthologist at the Calcutta School of Tropical Medicine, had identified the fluke eggs as those of Eurytrema sp. With regard to the suspicion of these worms being causally connected with haematuria, the remarks of Datta (1936) made in his Annual Report



Haematuria areas in red.

may be quoted in extenso:--

"Since the pancreas of cattle in that area are usually found to be severely parasitised by a species of Eurytrema worms, it was considered necessary to ascertain if these flukes had any role to play in the causation of haematuria. A number of affected bladders from this farm which were available for examination, failed to reveal any histological lesions indicative of helminthiasis. Haematuria cattle of the Garhwal and Kumaon hills have been repeatedly examined for the presence of pancreatic flukes with negative results. On the basis of the above findings and the existing knowledge that even in heavy infestations with known species of Eurytrema, an interstitial pancreatitis and debility are the only deleterious effects evinced, no etiological connection of these parasites with haematuria seems possible."

(a) Actual Localities.

The disease is known to occur both in the north and south of India. As the attached map of India (p.97) will show, its distribution in the north covers a somewhat wide area on the Himalayan ranges, while in the south it is restricted to the Nilgiri hills and Coorg. Other hilly tracts apparently similar to the haematuria localities exist in India, but



they are said to be free. The disease occurs frequently in the Kulu Valley, in the Punjab, in the Garhwal hills, and only occasional cases are encountered in the Kumaon ranges in the United Provinces. In the Bengal Presidency, the disease occurs in the Darjeeling and surrounding districts, including the Sikkim State.

From time to time field investigations were carried out by the writer in the actual enzootic areas. Attempts were made to obtain as much information as possible regarding the incidence, with particular reference to the following points:--

- (1) Regional distribution of the disease in each district.
- (2) Approximate elevation of affected villages.
- (3) What are the nature of vegetation and grazing, water supply and methods of husbandry? Are any common features invariably associated with enzootic areas?
- (4) Can any villager owning haematuria cattle recollect how and when the first case occurred in his farm, also whether any connection with the introduction of a new animal into the herd has been suspected?
- (5) Whether cases have been sporadic, or any increase in the proportion of affected animals in the herd has been observed?
- (6) Have any other species of animals housed and fed with affected cattle, any buffalo or ovines, been noticed to develop the disease?
- (7) What is the earliest age of an animal at which the disease was manifested for the first time?



(8) Any facts pointing to transmission taking place by means of contagion?

(9) Any difference in the feeding and watering of animals in the affected and non-affected farms in the same locality?

As a result of the above enquiries, on the whole no very significant data, clearly pointing to one factor or the other, has been elicited. In and around the Darjeeling area the disease is reported to occur between the elevations of 1000 to 8000 ft. above sea level, and an ideal enzootic locality appeared to be more or less level country with scanty fodder. An enquiry in two circles (Chamoli and Patti Dhagoli) of the Garhwal district showed that the disease occurs practically in every part of the high hills except the valleys. The approximate elevation of the affected villages in the former circle is about 3000 to 4000 ft., while in the latter the elevation is about 7000 ft. The water supply and method of animal husbandry were similar in the different localities, and though the nature of vegetation and grazing was roughly of the same kind, it seemed quite possible that plants which occurred in one locality were absent from another. Villagers were not able to recollect when and how the first case occurred, and no relation was brought out between the introduction of new cattle and the commencement of fresh

cases in a herd. In most places cases continued to be sporadic, though at times the number of cases in a herd was considerably more than at other times. Individual farmers had not noticed any obvious increase with the passage of time. So far only cattle have shown the disease, but the possibility of buffaloes contracting it has been suggested. No special age susceptibility has been observed, but perhaps more cases have come to notice between the ages of  $3\frac{1}{2}$  to 4 years or at puberty. As compared with the earlier cases, the degree of severity of the disease, or its course in recent cases, has not differed to any appreciable extent. An affected animal may survive for about six months to two years, or may linger for longer periods in spite of the active disease. If the affected animals are transferred from the enzootic areas to hotter climates, or when they are worked, the disease appears to be aggravated. In females the symptoms are somewhat relieved after parturition. As compared with those of the animals from non-affected farms, the feeding and watering of affected animals have shown no appreciable difference. In a farm one or two cases crop up now and again, and these eventually die. Similarly, other cases occur, but all the cattle in an affected farm do not show haematuria. It is clear that the disease

is fairly widespread, as cases have been encountered in many villages in the remote interior of the hills which happened to be investigated. The disease causes serious loss as in the aggregate a large number of cases occurs in each locality. Regarding the Nilgiri and Kumaon hills, much information as to the exact distribution and extent of the disease is not available. Generally speaking, it may be said to occur at an elevation of 4000-5000 ft. in the Nilgiri area, while the relative area in the Kumaon hills is about 6000-8000 ft. high. These Indian enzootic areas possess some of the characters of the affected farms elsewhere. The pastures in the Darjeeling and Nilgiri districts were excessively moist when visited, though perhaps they could not be considered water-logged. The rainfall is very frequent, and it is important to note that the atmosphere is surcharged with considerable amounts of moisture and mist most of the time. The relative humidity is high. The disease has not yet been reported from the hills of Cherrapunjee in Assam, which is reputed to have the largest rainfall in India, and perhaps in the world.

The geochemical aspect of the disease has not so far been studied in India, but an attempt has been made to ascertain if any common factors could be

suggested from the information already in the possession of the Geological Survey of India, regarding the well defined enzootic areas in India. The available information, which was kindly supplied by the Department, shows that the Indian areas are so large, and the geology for the most part so variable, that it is difficult to determine any common factor that might be responsible for the disease. The following, however, is a brief resume of the types of rocks to be found in these areas:--

Kulu. No modern geological map of this area is available. Roughly speaking, the lower or south-west corner of Kulu (i.e. approximately from Larji to Bhuntar at the confluence of the Parbati and Beas rivers, and from Larji south-east to Banjar and the adjacent areas) is composed in the main of slates and limestones with perhaps some quartzites. The rest of Kulu, so far as is known, consists of granite, gneiss and mica-schist.

Kumaon and Garhwal hills. These are made up of a variety of rocks, but modern maps are only available for a small part of the area. In general, it may be said that the inner hills are composed of mica-schist, gneiss and granite, the outer hills of slate and limestone, while a belt of tertiary rocks, composed mainly of sandstones and shales, forms the hills immediately bordering the plains.



Plate No. 1

A few haematuria cases in an upland farm in the Darjeeling district, situated at an elevation of about 6,000 ft.. Atmosphere surcharged with moisture and mist most of the time. Sudden drops in temperature.

Darjeeling district and Sikkim State. Roughly speaking, the higher country is composed of granites, gneisses and mica-schists, while the lower country, forming the valleys of the Teesta and its tributaries, is composed of slates, phyllites and quartzites. Along the southern margin of the hills, bordering the plains, there occur sandstones, shales and conglomerates.

Nilgiri hills. The rocks of these hills are mostly charnockites which, for the purpose of this investigation, may be regarded as granites. They are very deeply weathered to a variety of laterite, in which are set relics of the fresh charnockite. In certain parts of these hills, mostly above 6000 ft., peat is said to occur, but we have no detailed information.

From the above resume of the geology of these districts, it will be seen that, except in a very general way in the Himalayan areas, there is no common factor apparent in the geology. As already stated, however, the areas in question are so large that there is inevitably included in each area a considerable variety of geology. If, however, the exact localities where these cases are known to occur are studied, it might then be possible to give a more helpful opinion in the matter.

The above-stated facts regarding the rocks of these areas relate, of course, to the solid geology. Above this comes generally a sub-soil and soil which is derived primarily from the rocks below, but may be greatly modified by local climatic conditions. According to the report, no detailed information regarding the soils of these areas can be given, except to state that in the Himalayas the soils are very new, while in the Nilgiris they are matured.

A very general survey of the soils of India has been made by Wadia, Krishnan and Mukerjee (1935). Although this is more comprehensive and informative than any previously attempted, it fails to provide the precise details and accurate boundaries between different general types of soils that could be profitably correlated with the haematuria localities. The most that can be said is that the hillsides forming haematuria localities are humid regions, covered with thickly wooded and typical coniferous forests.

(b) Incidence of the Disease.

Seasonal.

The dates on which the symptom of haematuria was noticed for the first time, have been recorded as a routine, in every case of the disease occurring over a period of years in establishments situated in three localities in India. Attempts were made at

first to follow up each case as it was encountered, to determine if attacks of haematuria showed any seasonal variations with regard to severity, but as experience accumulated this line of approach had to be given up. Exact records of the monthly incidence of observed first attacks in two large Indian dairy farms, situated in two widely separated parts of the country, are summarised below, but it will be observed that for the reasons already explained, no clear conclusions are justified from these data, though according to the current belief in India, most cases should appear in the hot season, less in the rains, and least in the winter.

<u>Month</u>	<u>Darjeeling district</u>	<u>Nilgiri hills</u>	<u>Mukteswar</u>
January	4	4	1
February	4	3	3
March	1	2	2
April	3	1	2
May	4	0	0
June	1	12	2
July	5	5	1
August	3	1	2
September	4	2	1
October	7	0	1
November	1	7	3
December	1	3	1



Age Incidence.

<u>Age</u>	<u>Darjeeling district</u>	<u>Nilgiri hills</u>	<u>Mukteswar</u>
Under 6 months	2	-	-
Between 6 months and 1 year	1	-	-
Between 1 year and 2 years	7	-	-
Between 2 years and 3 years	12	2	-
Between 3 years and 4 years	5	5	-
Above 4 years	13	28	Majority at above 4 years

It may be added here that among the last group in the Darjeeling and Nilgiri animals, the largest number of cases occurred among the age-group 4-5 years, while at Mukteswar the largest number belonged to the 5-6 years' age-group and above.

Regarding the age incidence in other countries, a few representative data are cited below.

According to Hadwen, the average age at which animals become affected is 6 years. Lienaux states that the disease occurs in all ages of animals, but usually not in suckling calves. He admits that the disease may be exhibited by calves 3 to 4 months old, but only rarely. In Moussu's experience, the disease

is very rare in young animals and is exceptional before the age of  $2\frac{1}{2}$  to 3 years. In Ireland the disease is seen in aged animals only, frequently 8 to 10 years old, and occasionally 4 to 6 years. In no case has the disease been noticed in younger animals in Ireland.

Yearly Incidence.

<u>Year</u>	<u>Darjeeling district</u>	<u>Nilgiri hills</u>
1926	-	5
1927	-	1
1928	3	1
1929	4	7
1930	4	10
1931	4	5
1932	9	6
1933	11	2
1934	17	0
1935	5	2
1936	Not available	1

Sex Incidence.

No Indian data are available in this connection. The farms in which the disease has received the most attention, and where records are maintained, have

consisted almost wholly of dairy cows, while at the Mukteswar Institute only bulls are kept for experimental purposes, with the exception of a few dairy animals required for supplying milk to the Institute staff.

The position in literature has been somewhat similar. For instance, in Craig and Kehoe's (1925) experience, haematuria in Ireland was reported in cows only. Lienaux (1919), however, observes that no difference in incidence is seen in the sexes. Similarly, according to Moussu, the disease affects the male as frequently as the female animal.

One difference, however, exists, in that complications due to obstruction in the urinary passages are more frequently seen in the males, due to anatomical reasons.

#### Incidence in herds.

The actual percentage incidence in a herd is not definitely known. The figures that are available cannot be much relied upon. Experience shows that in some cases lesions may be present in the bladder for many years without obvious symptoms of haematuria being noticeable. The difficulty that is associated with the determination of the extent of the disease in a herd, can be well appreciated from the following

experience of Bull, Dickenson and Dann (1932),  
quoted in extenso:--

"On one such red water farm of nineteen cows, claimed by the owner to be free from the disease, five showed red blood corpuscles on microscopic examination, but two months later all showed red cells, and after a further period of four months, fifteen of the twenty-four showed red cells on microscopic examination of the urine."

It may be stated, however, that in India a greater susceptibility to the disease has been seen in imported breeds and in cross-breds than in the local breeds in the same herd. The disease is present among Siri and Nepali cattle in Sikkim. The incidence of the disease varies considerably in different localities and sometimes in neighbouring herds. In Darjeeling as many as 50% cattle in a herd have at times been actually passing blood, while the incidence at the Wellington Farm in the south of India has been less, but the incidence in the Kumaon hills has been the least of all. Similarly, in other countries relatively few cases have occurred in some herds over long periods, while in others the majority of cattle have become affected sooner or later. For instance, in one German herd all the twenty-two cattle contracted the disease, while in Bulgaria



80-90% in a single community may be affected, or only 10% of the total population in certain French cantons may contract the disease. Similarly, it was observed in New Zealand that out of a herd of twenty-six cows, there was a loss of thirteen during five years, i.e. a loss of about 10% per annum.

(c) Species of Animals Affected.

This disease is primarily an affection of the bovines. All the usual breeds of cattle are susceptible, and according to workers everywhere, young animals do not usually develop the disease. Miyamoto observed a number of cases among buffaloes in the Island of Formosa, while Avril states that the disease affects the horse and the pig as well. A form of chronic haematuria with lesions similar to those of the bovine disease, has also been encountered among sows in Ireland.

In India the writer has found a highly suggestive form of cystitis in the buffalo, and in the wild sambhur (Cervus unicolor) of the Himalayas.

(d) "Incubation Period".

Of the animals referred to above, it is somewhat important to note that two calves at Kalimpong developed the disease naturally in about the same time, viz. at the age of six months and nine days, and

five months and twenty-three days, while the previous observation of Lienaux shows that even younger calves may be affected. This observation would suggest that under natural conditions in an affected herd, the disease can be manifested within the minimum period of about six months or even three to four months. Again, the observation was made at the same affected farm in Darjeeling, that three imported animals above four years of age contracted haematuria after a stay at the farm for varying periods ranging from two years, three years and ten months, and six years. No clear idea regarding the incubation period could therefore be formed.

IV. (a) CLINICAL SYMPTOMS.

There is nothing very noteworthy in the literature relating to the clinical symptoms of this malady, excepting that the symptoms and course of the disease, as described from different countries, are practically completely identical. It has been known in universal experience that there need be no symptom other than the passage of blood most of the time, or until complications have supervened. The disease has been named essential haematuria by the French, and the apparently contradictory term, symptomless haematuria, of human medicine, refers to a similar syndrome. Though it may seem obvious, it is just as well to make the general statement that the clinical symptoms of the disease vary upon whether it is in the early stages, or well established and chronic, or whether obstructions and secondary infections have complicated the picture. The disease may, for convenience, be divided into the three stages, the pre-clinical, clinical, and the stage of complication. The first stage has been overlooked by most workers, and the last stage may be considerably shortened, as a result of an accident or other complication somewhat suddenly terminating life. An average observer is, therefore, frequently left

only with the clinical stage upon which to make his observations.

In the early stages, which are usually uncomplicated, systemic disturbance of any kind is not to be seen. There does not appear to be any rise of temperature or loss of body weight. The processes of feeding, rumination and other functions are continued unchanged. Affected animals look happy and bright, feed well, and apart from the character of the urine, there is no other abnormality to be noticed. At the present state of knowledge, it is not possible to specify the exact duration of the pre-clinical stage, which no doubt varies from case to case, but the writer's experience would seem to indicate that it may be as short as three to six months and as long as even four to six years. In such places in the haematuria localities as provide facilities for the making of large numbers of routine post-mortem examinations upon apparently healthy animals, (as during meat inspection in a slaughter house, or in the rare instance of disease investigation in such a locality), cases representing the pre-clinical stage are to be encountered. It can thus be found that the disease process in the viscera may progress to a considerable extent in animals, though from the standpoint of symptomatology the



disease does not appear to exist or even to show evidence of having already commenced. The blood corpuscles must attain a sufficiently high proportion of the urine, before the colour can be distinctly abnormal to attract attention. It can now be added that in the earlier stages only serum exudes into the urine, the cellular deposit is greatly increased making it translucent, or red blood corpuscles are added in such numbers as do not make the urine distinctly red. Unless the urine is collected and allowed to stand in a glass vessel, there is little likelihood of an early commencing case being detected in life. On suspicions having been aroused, however, and this is only likely to happen in a badly affected herd, the diagnosis of the disease by the microscopic examination of urinary sediments, or by the application of chemical tests for blood, presents no difficulty. An experience of Bankier may be quoted here in his own words: "Cases of the disease have been observed in which haematuria was noticed on one day only, many months elapsing before the recurrence took place."

The onset of haematuria symptoms is thus insidious and is characterised by a slow and progressive course with frequent intermissions of variable duration, extending from a few weeks to months.

Frequent micturition with blood passed at the end or throughout the act is seen, and mild symptoms of urinary colic may appear. There does not appear to be much difference in the total quantity of the urine passed. When the loss of blood has reached a certain limit, the haemorrhagic urine tends to coagulate on the ground, or even inside the bladder of the affected animal. The colour of the urine may be pale pink or bright red, depending upon the quantity of blood passed, or be dirty brown, or even smoky in complicated cases. Muscular exercise and straining on the animal's part tend to increase the haemorrhage. Occasional pigmentation of the urine has been seen by Hadwen in contaminated specimens, which he attempts to explain as a post-haemorrhagic haemoglobinuria. The passage of blood may cease suddenly or by degrees, only to reappear after a variable period in the same erratic manner. This process is repeated till the animal finally succumbs to extreme anaemia and debility, or to other complications such as severe internal haemorrhage into the bladder, hydronephrosis, uraemic poisoning, or super-added infections of the urino-genital system (cystitis, pyelonephritis), and perhaps also of the alimentary tract. There are also cases which run on an apparently benign course without any marked

symptoms of ill-health being exhibited, which quite suddenly terminate fatally, due to haemorrhage or to bursting of the bladder wall. The occurrence of diarrhoea in the late stages of haematuria has been mentioned by a few workers, but no causal relationship between haematuria and the development of actual diarrhoea or the passing of semi-solid faeces mixed with considerable amounts of mucus, appears to have been suspected. Although the brunt of the lethal effects of the disease haematuria is borne by the urinary system, it is important to determine whether other internal organs share in the untoward effects, though not quite to the same extent as the bladder or the kidneys. The majority of workers have regarded the disease as a purely bladder affection (Haematuria vesicalis), though they admit that at a late stage the kidneys may be involved, due to an ascending infection or to an obstruction in the urinary passages. The present author's experience regarding the extent of visceral involvement will be discussed under the pathological anatomy of the disease. In the uncomplicated early cases there is no marked evidence of any serious kidney involvement including oedema, rise of blood pressure, or the syndrome of renal colic, etc. Curiously enough, some animals live for years in apparently good

health excepting for the emission of blood in the urine. Generally the disease lasts for months and years, and in the experience of Moussu (1905) an animal aged twenty-eight years had been suffering for as many as twenty years. From the recurrence, intermittence and the prolonged course, it appears that the pathological process in the bladder (or in the animal's system) runs almost parallel to, or concurrently with the recuperative mechanism of the animal's body. The disease has been reported in young calves between the ages of three to six months, and the adult cattle exhibit the symptoms only after grazing a number of years on an affected farm-pasture. As regards animals at the Mukteswar Institute, history cards of each and every animal entering the estate are maintained regularly, starting from the day of birth, or of purchase from the surrounding hills, to the final disposal at the post-mortem room, either following natural death or destruction. From a careful scrutiny of the cards of the animals that were found to be affected either in life or at post-mortem examination, the period that elapsed between the purchase and the detection of haematuria has found to be short. From a knowledge of the protracted course of the disease, it appears reasonable to conclude that the majority of the hill bulls



found to be affected with haematuria at the Institute bring with them a pre-existing disease in their system, rather than contract the disease there. The percentage of animals found affected among the total population is approximately five per cent. In a previous paper (Datta, 1934) the remark was made that there did not appear to be any reason why the disease should not be contracted at Mukteswar, and why the place should enjoy any immunity. After a prolonged search for so many years, the writer was successful in 1936-1937 in establishing for the first time the occurrence of the disease in two locally bred animals. It was noted that both of the animals were about six years of age when they exhibited the symptoms of haematuria. Further, it has been observed there on several occasions that a number of cases invariably exhibit exacerbation of haematuria with each snowfall, and many cases which terminated fatally showed a gradual and continued fall of bodily temperature for a few days till death actually supervened.

In most exceptional cases and only in the early stages, a permanent cure may take place, spontaneously after the food and watering conditions have been changed, as by the transfer to a free locality or by therapeutic measures. Authorities exist who would

not accept that the disease is curable under any conditions, and argue that where such claims are made the actual state of the bladder has not yet been ascertained and that the affected animals have merely passed into the quiescent stage of the disease.

There are notably few workers who have, however, recorded the possibility of a cure being accomplished by the transfer of animals to plains stations or other places known to be free from the complaint. Once the disease is well established, as in a chronic case, there does not appear to be any possibility of a cure, and in this view every worker is definitely in agreement. This point of amenability to treatment will be developed further under control measures, but the present writer has observed an aggravation of the disease on several occasions when affected cases were transferred to hot climates.

Authorities are equally divergent on the question of whether the disease is contagious or not. It is reported from Germany, as well as from India, that owners of haematuria farms know since the oldest times that the disease is somehow transmitted from animal to animal. Lienaux (1919), however, questions that if the disease were contagious, one could hardly explain its enzootic distribution, and mentions that in the general opinion of the farmers in his country,

the disease is not such. Similarly, the observation has been made that on the transfer of affected cattle to non-haematuria districts, as from Darjeeling to Calcutta or from the Wellington Farm in the Nilgiris to Bangalore, the disease did not spread to the cattle already in the local herds, or develop in the apparently healthy animals that accompanied the haematuria cases. Lockett in Jamaica, and Bankier in Canada, also record that the disease has not been known to be transmitted to cattle in non-haematuria districts by the introduction of affected animals. In Ireland the disease occurs singly, but occasionally two or three cows in a farm may be affected at about the same time. Case (1911), however, mentions that in his country the disease is suspected to have been introduced with cattle imported from Australia and New Zealand.

#### IV (b) CLINICAL AND CHEMICAL STUDIES.

In the earlier part of the investigations, arrangements were made to collect full clinical details regarding the disease, including the taking of weekly body weights, daily temperature records, observing the frequency of urination, estimating the total quantities of the urine passed, etc., but no significant results were obtained. A method was improvised by the use of graduated test tubes to approximate the proportion of the admixture of blood cells and clots to a fixed amount of the urine passed. The total amount of blood passed in a 24 hour period was roughly estimated, and this varied considerably from animal to animal or in the same animal. In the stage of acute haemorrhage the amount varied from 200 c.c. to even 1000 c.c. or more. Blood smears were examined regularly as a routine from the commencement of observations upon individual cases till death occurred either naturally or through destruction for experimental purposes. Samples of urine, collected from each case with or without the use of the catheter, and post-mortem material as it became available, were subjected to cultural examination.



In order to eliminate protozoal haemoglobinuria, due to Babesia bigeminum, which is readily cured with injections of trypan blue, experimental treatment with this specific drug has been carried out in all the first cases studied. The benzidine test for the presence of haemolysed blood in samples of freshly collected urine, from which the centrifuged deposits had been removed, was carried out. The presence of bovine piroplasmosis was thus finally eliminated. By centrifugalising the urine and faeces from uncomplicated haematuria cases, and by means of the sugar floatation technique of Sheather (1923), (which is employed for the detection of coccidial oocysts and helminthic ova), repeated examinations were made, and these possibilities were similarly consistently negatived. Repeated attempts to culture any protozoan organism from urinary sediments by the use of a variety of media including 2%-5% potassium bichromate solution and hay infusions have given negative results. Bacteriological examination has been made upon both solid and liquid media, employing both aerobic and anaerobic methods. As opportunities occurred, careful post-mortem examinations were made in order to discover if the lesions in any other organs excepting those of the urinogenital system could be correlated with the passage of blood in

the urine. At the time the probability appeared to be that "the cause of the disease was an irritant chemical substance elaborated from the excretory products in the urine while the urine was stationary in the bladder." The investigation of the disease on the above lines continued, but it was increasingly felt that unless more information on some specific factors relating to the extravasation of blood from the bladder was available, the study of the disease was not likely to be productive of anything but negative data, and a critical examination of the possibilities in connection with each of the causes suggested from time to time in literature was made.

At that time, as already mentioned, Hadwen's was the only work which not only claimed to have reproduced the disease experimentally, but which was generally held to be true. Detroye's experimental work had come under the cloud of discredit. The views elaborated by Hadwen, that the disease resulted from the ingestion of oxalic-acid-bearing plants, had a very serious limitation. The fact that Hadwen admitted as having failed to secure in the affected farms and localities oxalic-acid-bearing plants in sufficient amounts for carrying out feeding tests upon experimental animals, (he had had to use commercial oxalic acid and oxalates instead), showed

that there existed no plausible alternative environmental conditions which could enable susceptible animals to obtain the required toxic amounts of such acid-bearing forage. Doubts regarding Hadwen's contention were thus expressed by the writer (Datta, 1931), who attempted to provide the only other possible explanation of the existence of oxalate crystals in the urine of affected cases. It seemed reasonable to argue that the cattle suffering from the disease do not feed upon such unnatural forage and fruits in excessive amounts as are known to contain much oxalic acid, e.g. rhubarb, spinach, tomato, apples, lettuce, grapes, cabbage and the common Indian sorrel, amrul, (Oxalis carinculata). Further, previous to Hadwen's researches, cases of experimental oxalic acid poisoning had already been studied. Servonat and Roubier (1911) showed how the poison localised in the various organs, and Chieri and Frohlich (1911) showed that a nerve excitability resulted. Datta (1931) was therefore led to elaborate as a working hypothesis, a methoblic theory suggesting that from the basis of existing biochemical knowledge an endogenous production of oxalic acid, due to defective elimination, could be implicated. As a result of Hadwen's work, the subject of oxalic acid poisoning has been studied

successively by a number of workers, Craig & Kehoe (1921), Kalkus & Sawyer (1924), Rost (1926), Bull (1929), Steyn (1933), and Jacoby & Friedel (1933). The theory has now been finally disposed of as untenable, though even as late as 1931 the Veterinary Bulletin made a statement to the opposite effect.

Therefore as the theoretical considerations of biochemical knowledge suggested, it was thought advisable to determine whether there was in the animal system any stagnancy of oxalic acid or other allied derivative, such as parabanic acid (oxalylurea) of metabolic origin, and also whether a deficiency of calcium or other metallic constituents of normal body tissues or any haemophilia existed, which could account for the tendency to the extravasation of blood seen in the living animal.

#### Coagulation Time of Blood.

Since in a few cases which died of long standing haematuria, the blood in the body was found to be still in a fluid condition several hours after death and with little tendency to clot formation being apparent, attempts were also made to test the coagulability of the blood. Various methods of determining the coagulation time of blood were tried but variable results were obtained (vide table No. I. H.B.1960). After preliminary trials with drops of



blood collected from the jugular vein, as also from the ear vein of affected cases, being put directly on glass slides or kept in corked and open phials, had been made, the loop method of Inchley (1921) was chosen as the most suitable working method. Curiously, the same average clotting time of eleven minutes was observed in all the animals examined (H.B.1168, 1190, 1960, 949), irrespective of whether they were in the active or quiescent stage with regard to the passage of bloody urine. The actual data are summarised below:--

THE COAGULATION TIME OF BLOOD DURING THE PERIOD  
WHEN BLOOD IS PASSED IN URINE AND WHEN IT IS NOT.

H. Bull No. 1960.

Haematuria from 15-7-30 to 12-4-31.  
 Urine normal from 13-4-31 to 3-5-31.  
 Haematuria from 4-5-31 to date.

3 grammes of Calcium lactate were given in feed morning and evening from 11-10-30.

Different methods were employed.

Date	Wt.the same day or two days later	Clotting time	Blood in urine	Temperature F°	
				Morn.	Even.
11-10-30	355 lbs.	33 min.	3.5%	101.4	101.8
3 drachm doses of Calcium lactate given thrice daily from 16-10-30.					
28-10-30	360 lbs.	46 min.	1%	102	102.8
4-11-30	370 "	25 min. 2 drops slide	1.5%	101.6	101
11-11-30	380 "	21 min. in bottle	2.75%	100.8	101.6
18-11-30	370 "	21 min. in bottle	3%	101	100.8
25-11-30	390 "	13 min. in bottle	3%	101	101
5-12-30	395 "	20 min.	3.5%	101.6	101.4

H. Bull No. 1960 (continued)Loop method.

Date	Wt.the same day or two days later	Clotting time	Blood in urine	Temperature F°	
				Morn.	Even.
11-12-30	395 lbs.	14 min.	3%	100.6	100.8
18-12-30	405 "	14 "	2%	101.2	101.6
25-12-30	405 "	20 "	1.5%	102.2	101.8
1-1-31	410 "	8 "	2%	101.2	101
8-1-31	415 "	11 "	1%	101	100.8
15-1-31	420 "	8 "	3%	101	101.2
22-1-31	415 "	11 "	1.5%	100.8	101.6
29-1-31	400 "	11 "	1%	101.8	101.4
5-2-31	400 "	11 "	1%	101.2	99.8
12-2-31	400 "	10 "	5%	101.4	101
19-2-31	405 "	12 "	1%	100.8	100
26-2-31	415 "	10 "	1%	100.8	100.4
5-3-31	410 "	10 "	1%	100.4	101.4
12-3-31	415 "	11 "	2%	100.2	101.4
19-3-31	425 "	9 "	5%	100.8	101.2
26-3-31	425 "	9 "	1%	101.4	101.8
2-4-31	420 "	10 "	5%	100.6	101.2
9-4-31	425 "	12 "	1%	100	101
16-4-31	425 "	12 "	0%	100.4	101.8
23-4-31	430 "	14 "	0%	101	101.8

Average 11 minutes since the adoption of loop method of 20 readings.

H. Bull No. 884.

25 c.c. Calcium chloride solution given by the mouth twice daily from 25-10-30.

Date	Weight	Clotting time (in bottle)	Blood in urine	Temperature	
				Morn.	Even.
18-10-30			received clotted		
19-10-30			2%		
20-10-30			2.5%		
21-10-30		20 min.		101	
22-10-30			3%	106	101
23-10-30			3.5%	99.8	100.8
24-10-30			received clotted	100	102.6
25-10-30			1%	101.6	102.8
26-10-30			received clotted	100	101.8
27-10-30			"	100.8	102.8
28-10-30	225 lbs.	24 min.	3%	101	103.8
29-10-30			2.5%	101	102
30-10-30			2.5%	101	102.8
31-10-30			received clotted	101.2	102
1-11-30			"	101.6	101.8
2-11-30			5%	101	101.8
3-11-30			received clotted	101	102.8
4-11-30	220 lbs.	10 min.	3%	100	102



H. Bull No. 884 (continued)

Date	Weight	Clotting time (in bottle)	Blood in urine	Temperature	
				Morn.	Even.
5-11-30	205 lbs.	9 min.	3%	100.4	102
6-11-30			4%	101	102.2
7-11-30			4.5%	100	102.4
8-11-30			3%	100.8	101.6
9-11-30			3%	101.6	102.6
10-11-30			3%	101	101.8
11-11-30			2.5%	101	101.8
12-11-30			3%	101	102
13-11-30			3%	101	102.8
14-11-30			3%	101.2	100.8
15-11-30			3%	100.8	102
16-11-30			received clotted	101.6	100
17-11-30			2.5%	100	100.2
18-11-30		18 min.	2%	99.8	99.8
19-11-30			3%	99	98.8
20-11-30			2%	98.6	100
21-11-30			3%	98.4	
		Average 16 minutes			

H. Bull No. 1168.

Passed blood in the urine from 11-12-30 to 17-3-31.

Urine was normal from 18-3-31 to date, with haematuria on 8-4-31 only.

The loop method of Inchley (1921) was used to determine the clotting time of blood.

Haematuria.

Date	Clotting time	Blood in urine	Temperature	
			Morn.	Even.
12-12-30	10 min.	2.5%	100.4	101.6
25-12-30	15 min.	2%	102	101.4
1-1-31	10 min.	1.5%	101	102
8-1-31	10 $\frac{1}{2}$ min.	1.5%	102.2	102.2
15-1-31	10 min.	2%	101.6	100.8
22-1-31	12 min.	1.5%	101.4	100.4
29-1-31	11 min.	2%(about)	100.6	99.8
5-2-31	10 min.	1.5%	99.6	100.2
12-2-31	10 min.	1.5%	100.8	101
19-2-31	11 min.	1%	99.2	100.4
26-2-31	10 min.	1.5%	99	100.6
5-3-31	10 min.	3%	100	101
12-3-31	11 min.	2%	99.8	100
Average 11 minutes				

H. Bull No. 1168 (continued)Quiescent stage.

Date	Clotting time	Blood in urine	Temperature	
			Morn.	Even.
19-3-31	9 min.	0	99.8	100.8
26-3-31	10 min.	0	101.4	101.8
2-4-31	10 min.	0	101	101.6
9-4-31	13 min.	1%	100	100.8
16-4-31	10 min.	0	101	101.4
24-4-31	10 min.	0	101.6	102
Average 10 minutes				

H. Bull No. 1190.

Passed blood in urine from 28-11-30 to 7-12-30.

The loop method of Inchley was used.

Normal.

Date	Clotting time	Blood in urine	Temperature	
			Morn.	Even.
14-12-30	9 min.	0	101.4	102
23-12-30	11 min.	0	100.8	100.8
30-12-30	13 min.	0	100	100.8
6-1-31	9 min.	0	100.4	100.2
13-1-31	7 min.	0	99.4	99.8
20-1-31	12 min.	0	99.6	100.6
27-1-31	12 min.	0	99.2	99.4
3-2-31	13 min.	0	100.6	101.2
10-2-31	12 min.	0	99.6	100.2
16-2-31	12 min.	0		
23-2-31	11 min.	0		
2-3-31	12 min.	0		
9-3-31	10 min.	0		
Average 11 minutes				



H. Bull No. 949.

Received 100 c.c. calcium chloride and sodium chloride solution intravenously once daily since 12-12-30.

Biochemical analysis made and coagulation time noted once every week. (Loop method).

Date	Weight	Clotting time	Blood in urine	Temperature	
				Morn.	Even.
5-12-30			2%	102	101
6-12-30			2%	101.6	101.4
7-12-30			2%	99	100
8-12-30			2%	98.6	102.4
9-12-30			2%	101.2	101.4
10-12-30			2.5%	98.4	100.4
11-12-30		14 min.	2%	99.8	100.4
12-12-30	315 lbs.		2%	99.4	101.8
13-12-30			2%	101	101.2
14-12-30			1%	100.8	101.4
15-12-30			2%	100.6	101.8
16-12-30			3%	101.8	102.8
17-12-30			3%	101.2	102
18-12-30			3%	102.2	100.8
19-12-30	325 lbs.	9 min.	3%	100.8	101.4
20-12-30			2.5%	100.6	101
21-12-30			2%	101.2	101.4
22-12-30			3%	101	100.8
23-12-30			3%	101.6	101.2

Date	Weight	Clotting time	Blood in urine	Temperature	
				Morn.	Even.
24-12-30	325 lbs.	16 min.	3%	99.4	101.2
25-12-30			2.5%	101.6	101
26-12-30			2.5%	101	100
27-12-30			3%	100.2	100.8
28-12-30			3%	100.8	101
29-12-30			2.5%	101.4	101
30-12-30			2%	100	100.8
31-12-30	320 lbs.	8 min.	2%	98.2	101
1-1-31			1.5%	100.4	101.6
2-1-31			1.5%	98.4	101
3-1-31				97.8	101.4
4-1-31			1.5%	102.2	102.2
5-1-31			1.5%	100.2	100.8
6-1-31			2%	100.4	100.2
7-1-31	320 lbs.	8 min.	2%	97	100.2
8-1-31			2%	100	99.6
9-1-31			2%	100	99.6
10-1-31			2%	98.2	99.2
11-1-31			3%	98	98.6
12-1-31			3%	99.2	98.2
13-1-31			2%	99.4	99.8
14-1-31	330 lbs.		2%	99.6	99.6
15-1-31			2%	99.2	101
16-1-31			3%	100.2	100.8

Date	Weight	Clotting time	Blood in urine	Temperature	
				Morn.	Even.
17-1-31		8 min.	2%	98.6	100.2
18-1-31			2%	99	99.8
19-1-31			3%	98.6	100.6
20-1-31			3%	101.2	101.8
21-1-31			2%	98.8	101.2
22-1-31	325 lbs.		2%	99.2	100.2
23-1-31			2%	100.6	99.8
24-1-31		10 min.	received clotted	98.6	100
25-1-31			"	100.6	99.8
26-1-31			"	99.6	98.2
27-1-31			"	98.2	99.2
28-1-31			"	99	99.4
29-1-31	315 lbs.		"	99	99.6
30-1-31			"	99.8	98
31-1-31		10 min.	"	98.6	98.2
1-2-31			"	98.4	100
2-2-31			"	98.6	99.4
3-2-31			"	98.6	98.6
4-2-31			1%	98	99
5-2-31	310 lbs.		received clotted	96	
Average $10\frac{3}{8}$ minutes					

### Urine Examination.

With regard to the urine itself, its specific gravity, reaction and composition were also studied. In the writer's experience the specific gravity of haematuria urine varied from 1020 to 1035 in different individuals, and from the observations made on several animals, the rise and fall in the specific gravity from time to time did not appear to bear any relation to the percentage of blood present in the urine at different intervals. (Table below). These figures compared favourably with those of Canadian and Australian workers.

### Specific Gravity of Urine.

Animal	Date	Blood in urine	Specific gravity
H.B. 1960	19-7-30	2%	1035
	25-7-30	5%	1030
	28-7-30	1%	1030
	2-8-30	1%	1020
	11-8-30	1%	1030
	27-8-30	Positive to benzidine test	1035
	1-9-30	"	1030
	6-9-30	1%	1030
	7-10-30	4.5%	1030



Specific Gravity of Urine (continued)

Animal	Date	Blood in urine	Specific gravity
H.B. 1960	13-10-30	5%	1035
	18-10-30	.75%	1030
H.B. 357	27-7-30	5%	1035
	29-7-30	5%	1030
	31-7-30	2.5%	1035
	3-8-30	3%	1035

The reaction of the urine in each case was found to be alkaline to litmus, phenol red and brom-thymol blue. Other workers also report the reaction of the urine to vary from pH 7.1 to 8.4.

Samples of urine from three cases were examined for the presence of soluble oxalates, sugar, etc., with negative results. Albumin was present, as expected. The result of chemical analysis on an average sample of haematuria urine from an untreated case is given below:--

The figures are per 100 c.c. of urine.

Amount of PO <sub>4</sub> (phosphates)	0.0148 gm.
" of calcium	0.024 gm.
" of albumin	0.060 gm.

Urea was not estimated.

Some authorities have reported that the urinary output of potassium is highest towards the end of summer and lowest in the spring, but were rather doubtful about any possible significance of this observation. According to Butozan's (1938) researches, no differences exist between the calcium and chlorine values of healthy and haematuria cases.

Octahedral crystals of calcium oxalate, which are insoluble in acetic acid but soluble in hydrochloric acid, are frequently found in the haematuria urine, but their number is very variable. Other crystalline forms are met with. Amorphous earthy phosphates are also present, and they are insoluble in alkalies but soluble in acetic acid. Hadwen (1917) states that the calcium oxalate crystals are more plentiful in the early stages of the disease, and these have been found in varying amounts by others. Gordon states that union takes place between oxalates and albuminous substances, and Roger observes that the number of crystals is not a true indication of the amount of oxalates present in the urine, particularly when much blood is passed.

When blood is present in microscopic quantities only, two chemical tests can be employed, viz. the benzidine test and the phenolphthalein test. Of these, the former was found to be very delicate and

was used as a routine measure on both naturally affected and experimental subjects.

#### Microscopic Examination of the Urine.

The examination of haematuria urine is likely to be somewhat confusing unless one has first acquainted oneself with what is expected to be found in the urine of healthy cattle in a particular place. The possibility of certain extraneous materials finding their way into the collected samples of urine, like the spores of pine cones, pine needles, types of pollen grains, and other vegetable matter, certain forage acari, etc. must be recognised. One must familiarise oneself with the appearance of the character and appearance of urinary sediments of healthy cattle, particularly after such sediments have been treated with the various reagents and stains that are to be employed in haematuria studies. Thus artefacts have to be guarded against in material treated with Caustic potash solution. Samples of the urine from healthy cattle are singularly free from any marked suspended deposits, and practically always they have been found to be bacteriologically sterile.

Sedimented or centrifuged/<sup>urinary</sup>deposits of haematuria cases may however be considerable. They are found to consist of crystals of urinary salts, various

types of tissue cells and casts, "iodophile and diffusion bodies", amoeboid leucocytes, and occasionally pus cells. No helminth parasites or their ova are detected. At first the urine is practically bacteria-free, but streptococci and diphtheroid organisms are frequently seen, particularly in long-standing cases. The proportion of the admixture of blood in the urine varies within wide limits. Only a few red corpuscles may be discharged into the urine, or bleeding may be copious and continuous. Minute flakes of blood clots may be present, or the total content of the bladder may form a gelatinous clot. When the bladder contents are composed mainly of extravasated blood and cells, a proper examination of the fluid becomes extremely painstaking, and if blood clots have already been formed, it becomes more so. Methods for the breaking down of the blood cells and clots have to be adopted, and though these methods are too drastic for certain parasites, fungi generally are not much interfered with. Fungi and various fungal structures are thus brought out clearly. In haematuria urine, fungal structures representing one or more stages are invariably present, and of these the conidia and mycelia are detected most readily. Actual conidiophore and perithecia, chlamydospores, (Plate Fig.I) and germinating "spores"/are also found in most cases



but repeated examinations over a length of time may be required before all the stages can be traced in each and every case. It may be noted that no previous worker has described or mentioned the occurrence of any fungal elements in haematuria urine. Comparatively large "bodies" or "cells" have, however, been seen by a number of investigators, some of whom have ignored them as degenerated epithelial cells, while others have interpreted them as "Coccidia." (Pl.8, Fig. I&II & Pl.9, Fig. I).

Examined under the warm stage of the microscope, pseudopodial activity of body tissue cells, as well as of certain parasites, may be evinced.

HAEMATOLOGICAL

Blood smears were collected from the peripheral circulation of a large number of animals over a length of time. These were stained by different methods and subjected to microscopic examination. In the early stages the blood appears to be normal, but in different other stages of the disease the picture is variable. The red blood corpuscles vary in their sizes, and irregular and wider areas devoid of haemoglobin may be seen in them. In short, the usual changes observed are those following haemorrhage and secondary anaemia. The extent of anaemic changes present does not appear to be any indication of the stage of the disease, though in extreme forms of anaemia the prognosis is no doubt grave. When stained with methylene blue and eosin the erythrocytes may show the normal bright red colour. With the progress of the disease a varying amount of admixture of minute blue staining granules (punctate degeneration), anisocytosis and incomplete staining may be seen. Blood smears collected peripherally or from the jugular vein do not reveal the presence of any parasites to which significance can be attached. Differential blood counts were made, but they did not reveal any significant change. The differential

leucocyte counts obtained are given below, for comparison with the normal figures given by Dimock and Thompson, and by Fraser:--

Differential Leucocyte Count  
in Haematuria Cases and in Healthy Animals.

	Polymorphs	Eosinophiles	Lymphocytes
Normals given by Dimock & Thompson	30.5%	13.15%	54.2%
Fraser's average	30.7%	11.2%	57.9%
Bull number (Mukteswar)			
74	34.5%	8.5%	57%
640	22%	6%	73%
428	27%	14%	62%
968	30.5%	6.5%	63.5%

Similar figures for haematuria cases have been recorded by Case (1911), who states that the differential counts of these cattle do not materially differ from those of normal cattle. His figures relate to five animals, and are quoted below:--

Animals	Polymorphs	Eosinophiles	Lymphocytes
1	29.7%	9.9%	58.7%
2	36.4%	8.8%	57.2%
3	14.8%	7.5%	76.6%
4	44.8%	6.5%	54.09%
5	31.0%	9.2%	58.8%

From the above data it may be noted that the blood picture in this disease is not distinctive or even suggestive. In progressive cases a reduction in the haemoglobin content of blood, as also in the number of red blood corpuscles, indicative of a secondary anaemia, is the only common feature. A mild or moderate degree of leucocytosis may be exhibited. Where pyogenic or other secondary infections have complicated the picture, a polymorphonuclear increase may be evident. Turc cells are present. The *Aspergillus* parasite has been recovered in culture from the jugular blood on several occasions. (Plate 48).

In order to ascertain whether there was any hyperacidity of serum in affected cases, the pH values of some healthy and eight haematuria subjects were determined colorimetrically, with the result that an average value of 7.6 was obtained for the



healthy, while in the case of diseased animals it varied between 7.3-7.8 and averaged 7.5. There was therefore no marked discrepancy, but whether a large proportion of neutral salts of acids was present was not determined.

The inorganic calcium in the blood serum of a number of healthy and affected hill bulls was studied. The figures for the haematuria animals are as follows:--

Animal no.	Serum calcium
H.B. 74	10.63 mgs. per 100 c.c. of serum
H.B.1960	10.40 " " " " " "
H.B. 884	9.99 " " " " " "
H.B. 949	9.50 " " " " " "
H.B. 972	9.50 " " " " " "

The corresponding average phosphorus content of the sera of haematuria subjects was found to be 4.4 mgs.

Further calcium was administered in various forms to affected cases, since it was desired to study what effect any resulting raised calcium content would have in counteracting any haemophilia that might exist. The administration, morning and evening, of calcium lactate (zihi doses, twice daily) in the feed, and of calcium chloride intravenously, did not

produce any marked difference in the coagulation of the blood in affected cases. The clotting time in one untreated clinical case, however, rose to 18 minutes a short time prior to death. Similarly, when the serum calcium was estimated in those haematuria subjects which were receiving calcium therapy, for purposes of comparison with those that were not, the calcium value showed only a small increase, but the phosphate value in the treated subject was decidedly on the increase. Another noticeable fact in these experiments was that, even during the period when as much as 5% of the urine passed by an animal consisted of red corpuscles, it was found to be putting on weight consistently. This gain in weight may be ascribed to the benefits of calcium therapy.

As a result of chemical estimation of the calcium and phosphorus content of some healthy and seventeen haematuria cases, Schlegel (1934) states that the figures from all the diseased animals are low compared to the normals of cattle. The calcium content of one of his cases is said to have dwindled down almost to zero. Contrary to this, analyses carried out at Mukteswar as late as a few hours before the death of an affected subject, have given practically normal figures. Besides, Butozan (1938) in Yugoslavia carried out similar calcium and chlorine

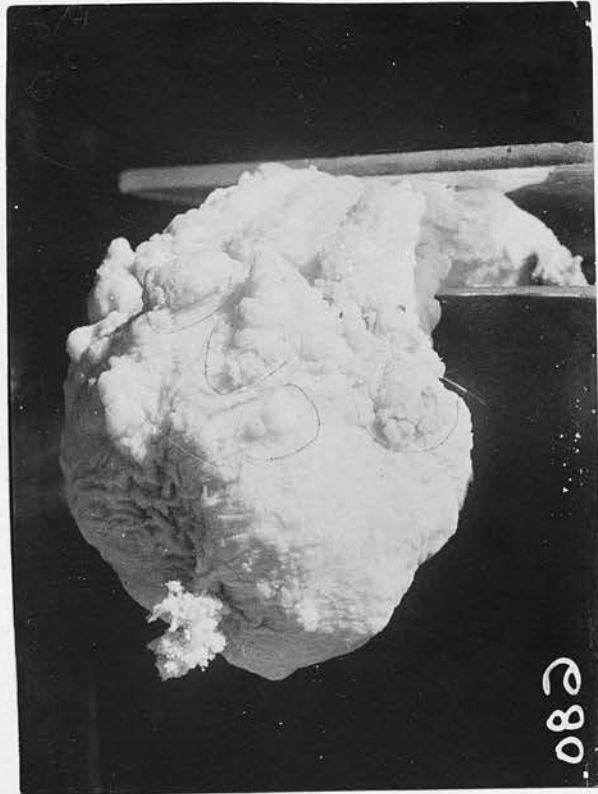
estimations of the blood serum of healthy and haematuria animals. He, too, was unable to detect any noteworthy difference in the values of the healthy and diseased animals. The definite reduction of the calcium value reported by Schlegel has therefore not been confirmed.

V. THE LESIONS.(a) Pathological Anatomy. (Macroscopic Lesions).

If an affected animal be subjected to a post-mortem examination, lesions of significance may not be usually 'seen' with the naked eye in many of the internal organs. Frequently hydatid cysts may be present in the lung, liver and elsewhere, and the common gross parasites like the liver flukes, Fasciola gigantea, Fasciola hepatica or Dicrocoelium dendriticum, may have produced marked lesions. In some areas in India these and other parasites severely parasitise animals, and hydatid cysts may be so numerous and extensive that very little of the liver or lung tissue may be left unaffected. The presence of such marked lesions creates a confusing situation in the mind of the examiner, so that any less differentiated lesions/<sup>of haematuria</sup> in these organs are obscured and readily passed over as having no connection with the obscure bladder disease under investigation.

The urinary bladder. The lesions that are produced in this organ vary considerably in degree. (Plates 2,3,41) Yet they are so typical and even so marked that little difficulty is experienced in detecting them. This remark does not apply to the earliest commencing

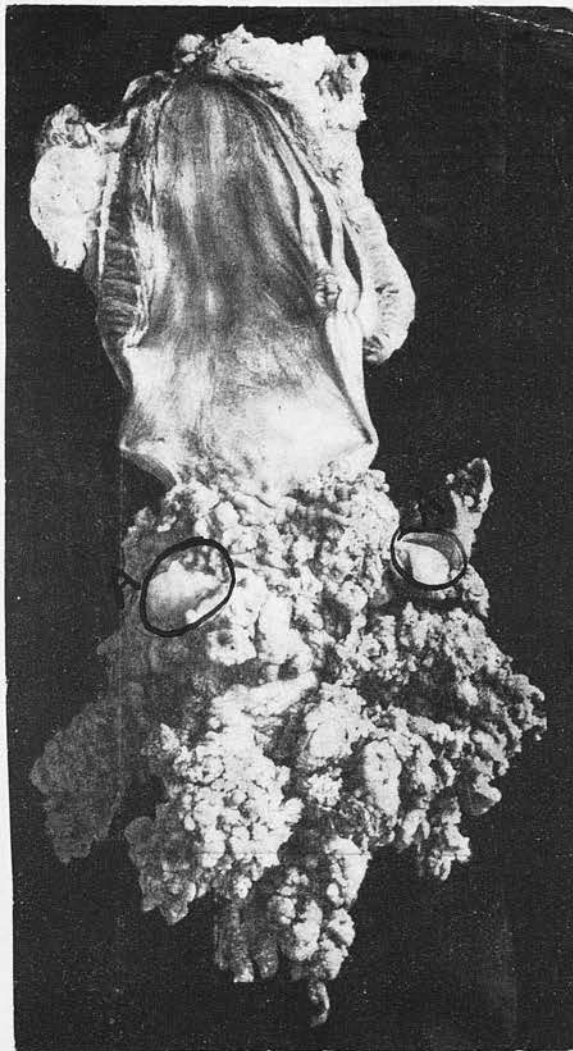


Plate No. 2

Urinary bladder. Natural case. Turned inside out. Note the pediculated soft papillomatous growth at the bottom, and the aggregations of somewhat solid-looking, broadbased 'warts' at the upper part of the organ.

lesions of the disease, which are situated in the submucosa or deeper down in the bladder wall, and are often missed. In fact the minute lesions of natural cases have repeatedly been missed by previous workers. Besides, workers have experienced considerable difficulty in interpreting correctly the changes observed in the viscera of experimental animals destroyed a short period after the commencement of the attempted transmission work.

The bladder may be empty, or filled with haemorrhagic urine which may contain flakes of clotted blood or sometimes of serum, or, as in the late stages, the total bladder contents may have partially or completely clotted. On eversion of the organ, the initial lesions may be found in any part of the bladder, but pronounced lesions predominate generally around the urethral opening, and to some extent in the basal and ventral part of the bladder. The earliest lesions may appear as only small patches of congestion and red dots of haemorrhage up to the size of a lentil. The differentiation by the naked eye alone of these from the "commoner garden" changes often seen in the post-mortem room, and which are of no significance, is by no means easy. The history of the animal and locality, and the simultaneous finding of obvious lesions, are no doubt helpful.

Plate No. 3

Urinary bladder. Protracted case. Incised to show the much branched cauliflower-like clusters filling a considerable part of the bladder cavity. Note the two pyriform mucoid cysts. Similar 'polyps' may be found in the kidney, and in the air sinuses of the skull. These have been experimentally reproduced with pure cultures of the haematuria *Aspergillus*.

The final confirmation, however, as will be seen later on, must depend upon the minute histological features. The mucous membrane may be shiny and smooth, though forming varying numbers of nodular elevations of different sizes. A gelatinous oedema of the submucosa, more marked and localised in the nodular lesions, may give a tense or taut appearance to the mucosa.<sup>(Plate 47)</sup> In places the mucosa may be dull, discoloured and irregular in appearance. The nodules may appear as only small yellowish pimples enclosed by a ring of congestion. In protracted cases much-branched cauliflower-like growths may be present, filling a comparatively large part of the bladder cavity. (Plate No. 3 & 41). The mucous membrane appears to be generally intact, apart from minute breaches here and there, which may be associated with haemorrhagic discoloration, necrotic areas and ulcers with or without adherent deposits of one kind or another, including even clots. The ulcers may be discrete. When confluent, they may form broad and elongated lesions with somewhat raised edges. When the overlying mucous membrane has given way, a process of continuous blood-letting goes on until the opportunity for clot formation arises. In some bladders the haemorrhage results from extensive eroded patches. The terms "warty" and "verrucose" have been employed



to describe the bladder growths of this disease.

These growths may be disposed individually, or may form closely set aggregations or clusters, some of which may be pediculated while the rest are sessile. (Plate 2)

Among the pediculated structures there may be present

(Plate 3, Fig. A.)  
a few pyriform mucoid cysts. The bladder wall may

be thickened uniformly, but this thickening may be

accentuated in the areas which carry the nodular

growths. If the bladder is sectioned with a knife

and its wall is closely examined, considerable variation

in the relative thickness of the different

coats may be apparent. The bladder wall may be

flaccid due to a loss of its normal elasticity, and

sometimes the wall may be considerably atrophied.

Generally the mucous and submucous coats appear to be

more haemorrhagic than the other parts. One peculiarity

of the bladder lesions appears to be the apparent

absence of any marked cicatrices. While this has

been an observation of general comment, no satisfactory

explanation has yet been put forward. Some

workers, like Hadwen and Schlegel, have sought an explanation

by assuming that the physiological process

of cicatrization has been upset (owing to oxalaemia,

or to deficiency of calcium or of pro-thrombin.)

To the present writer, however, the apparent absence

of cicatrix does not appear to form a special

characteristic of the disease, but would seem to be connected with the nature and structure of the bladder tissue itself. Another finding which has been repeatedly commented upon, is the absence of any clear relation between the extent of the bladder lesions with the degree of haemorrhage exhibited at any time. While on the one hand, animals passing large quantities of blood have been found, on immediate autopsical examination, to present inappreciable lesions, animals passing little or no blood have, on the other hand, exhibited very extensive growths. The finding of marked lesions in quiescent stages must appear incongruous, when compared with the lesions which are apparently insufficiently developed to account for the severe haemorrhage with which they have been found to be associated. This finding would seem to suggest that the polypoid growths tend to form when there is no direct outlet for the accumulated blood in the subjacent bladder tissue, while the existence of a direct opening of the mucosal and submucosal blood vessels into the bladder cavity, provides a ready exit for the blood to produce an active haemorrhage, but without appreciably stimulating any proliferative activity of the lining coats of the organ. In a few cases which were apparently "hale and hearty" till suddenly a fatal internal

haemorrhage or bursting of the bladder wall supervened, the writer found extensive necrosis involving large areas of the organ.<sup>(Plate 41)</sup> The lesions that are generally found in an average animal are varied, showing a gradation from minor changes to exuberant growths, so that quiescent lesions co-exist side by side with active changes in the same bladder. The lesions no doubt develop separately, though continuously. This offers an explanation for the remarkably intermittent and persistent character of haematuria.

In the literature, the disease has been considered to be an exclusively bladder affection. Marked changes in the kidneys and the ureter were observed even by some of the earliest workers, like Pichon and Sinoir, but these have not been regarded as forming part manifestations of the same disease. Even where a few workers have gone so far as to ascribe a significance to renal and ureteric lesions, they have considered them to be only of a secondary nature, due to an obstruction in the passage of the urine, or due to an infection with contaminative bacteria. Schlegel (1934), however, asserts that marked kidney lesions are invariably present. The correctness of this observation has been repeatedly confirmed in the present investigations.<sup>(Plate 42, Fig. 3&4)</sup> It is admitted that special care is necessary to be able to

recognise the kidney lesions with the (unaided) naked eye, but once the picture of the abnormality to be looked for is appreciated, the existence of lesions can be recognised without much difficulty.

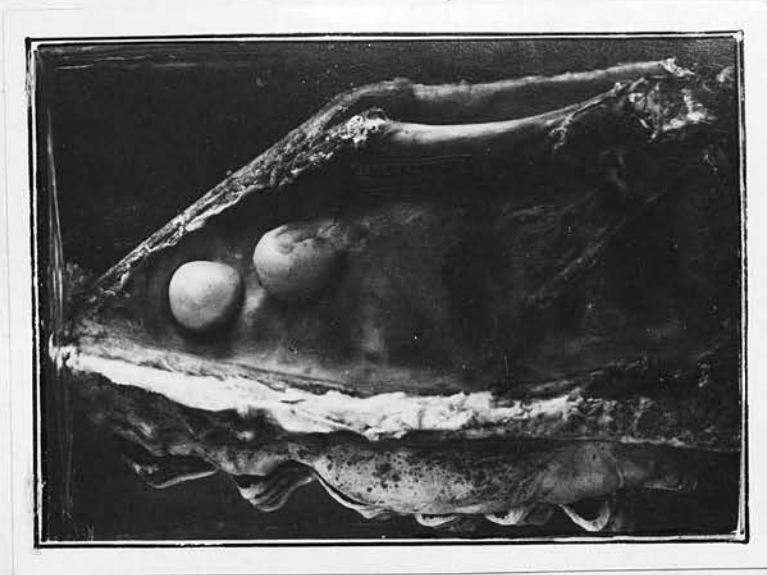
Regarding the actual lesions, the capsule may be partially, or to some extent completely adherent to the organ, making its stripping off somewhat difficult. The surface of the organ may show irregularities and contortions on the lobules of the organ. (Plate 43) Some of the parts may be contracted, while others are swollen and enlarged. Parts may appear haemorrhagic, while a yellowish or pale grey mottling may characterise other parts. Pimple-like elevations or split pea-sized depressed lesions may be seen. The organ may be flabby or tense. It may be enlarged or contracted, and fleshy and even hardened. Considerable amounts of fluid, composed of varying amounts of the urine and blood, may accumulate inside the organ, producing varying sizes of multiple cysts in the kidney, or a pronounced hydronephrosis involving the pelvis and the calyces of the kidney. The organ may be considerably hollowed out and its parenchyma flattened ("ballooned"), due to the great pressure exerted by the fluid accumulated inside it. When the organ is cut into, this varying amount of fluid escapes, and may be found to be only blood from the



congested and haemorrhagic organ, or actual haemorrhagic urine with yellowish flakes or blood clots. Mucoid cysts are found on the surface of the organ, as well as in the depth of the parenchyma. The material contained in the cysts is colourless or whitish, and may be gelatinous in consistence. Of the different regions, the medullary and the cortical portions may present a highly congestive appearance, and a considerable distortion of the calyces may be present. Both the kidneys show similar lesions, though there is some variation in their extents in the two organs, particularly where a state of compensatory hypertrophy exists. For instance, while one kidney is highly cystic or affected with advanced hydronephrosis, the other kidney may show relatively little of these changes.

Urinary calculi were detected in the ureters and kidneys of a number of clinical cases, and these on analysis were found to be composed mainly of calcium carbonate.

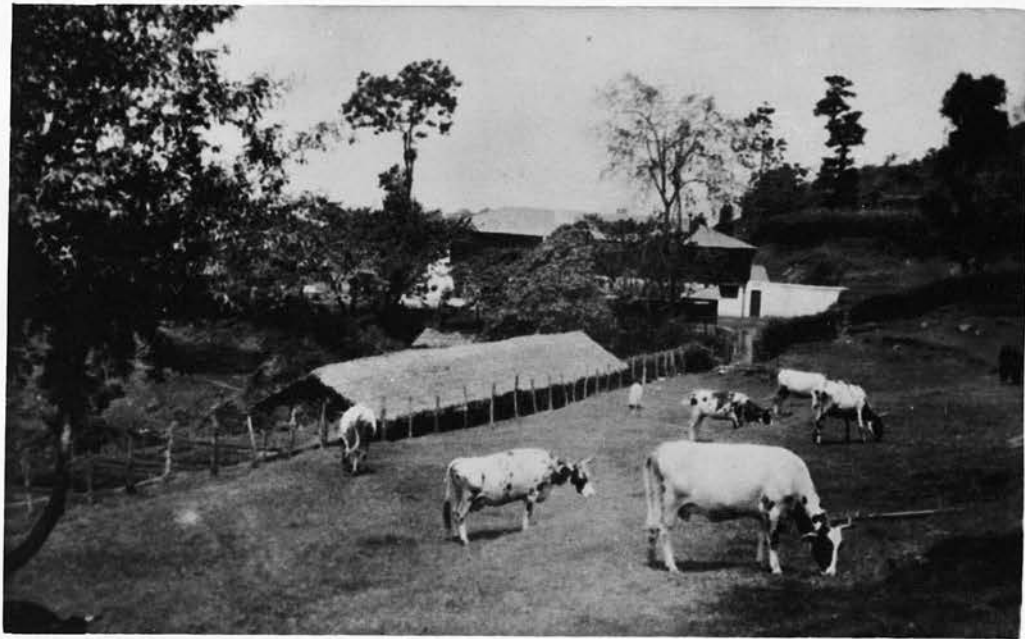
Among other organs often found affected is the liver. Lesions have already been reported by Hadwen, who states that occasionally the condition of the liver is not normal, and that in two or three cases there have been cancerous complications. Bull, Dickenson and Dann observe that the liver lesions

Plate No. 4

Air sinuses of the skull. Natural cases. Showing the mucoid cysts. Fungal fructifications have been repeatedly seen in the stained sections studied. The occurrence of such growths in this situation does not appear to have been recorded previously. These lesions have been reproduced in healthy cattle with pure cultures of the haematuria *Aspergillus*. cf. Plate 43, Fig. 9.

are relatively frequent, but like other workers, they also dismiss these lesions as perhaps unconnected with the disease. In the experience of the present writer, however, there has been no doubt about the identity of the liver lesions with those in the kidney or in the bladder. The lesions can be seen on the surface of the organ as multiple pimples or depressions of varying sizes, some of which are somewhat rectangular in shape. (Plate 42) Similar lesions may also be found on cutting into the parenchyma of the organ.

Turning to the other organs of a haematuria case, localised tubercule-like nodules may be found in the (Plate 7, Fig. II) lung, polypoid growths or minute haemorrhagic lesions may be present in the intestinal tract, mucoid cysts may be present in the air sinuses of the skull simulating those of the kidney and the urinary bladder, (Plate 4) and lesions may also be found in the mammary glands. Occasionally lesions may be detected by the naked eye even in other parts of the body, but this is not the invariable rule. The bronchial and mediastinal lymphatic glands, the mesenteric glands, and even the spleen, may be affected with the lesions, but it is not easy to detect them with the naked eye. Microscopically the lesions can be demonstrated without doubt. In late stages of the disease, the symptom of diarrhoea has been reported by a few workers, but

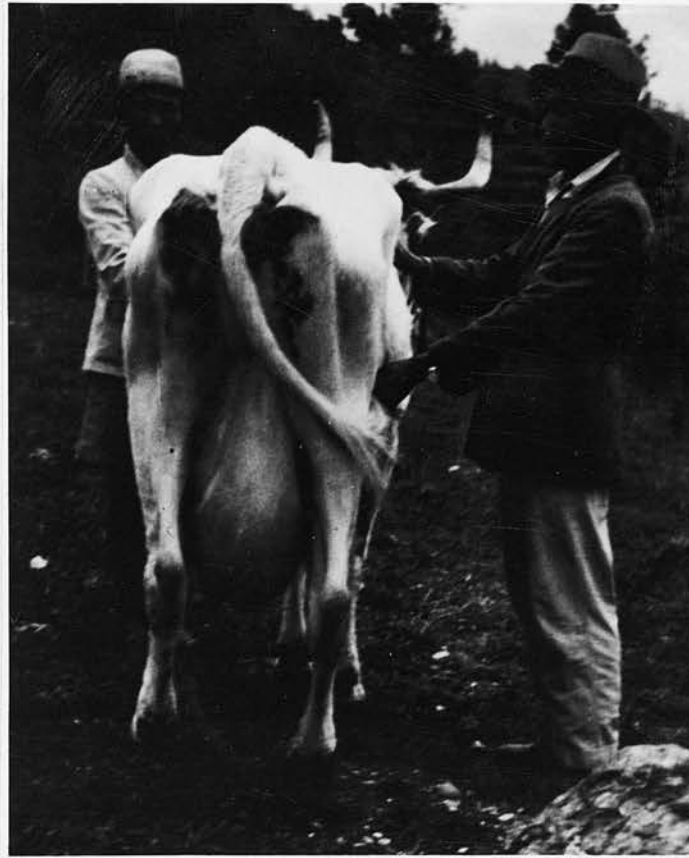
Plate No. 5

A few of the crossbred Ayrshire animals affected with haematuria, grazing in their home paddocks in Bengal. The paddocks are wet, the atmosphere is highly surcharged with moisture, thick mists are frequent, and the area is thickly wooded and contains typical coniferous forests. This description applies equally well to other affected areas in India.



it is noteworthy that no connection between this symptom and haematuria has been suggested by any one. Sub-epithelial haemorrhages and small ulcers are frequently present in the intestinal tract of cattle from a variety of causes. Therefore any possible connection of these lesions with haematuria cannot be hazarded without the help of a microscopic examination. In a number of cases, however, the intestinal lesions have been proved in these studies to be definitely causally connected with the bladder disease.

In 1936 the writer visited one of the worst(Plate 5) affected herds in his country, and availed himself of the opportunity to hold some post-mortem examinations there. He was rewarded by the finding of lesions of unprecedented generalised character, and in altogether new situations. Two chronic cases, each of six years' standing, were particularly noteworthy, since a crop of haemorrhagic growths, greatly resembling those of the urinary bladder, were found involving to various extents the ileum,<sup>(Plate 7)</sup> colon, and the ureters. In one case the ureters at their commencement in the renal pelvis were affected, while the udder had undergone an enormous enlargement with profound haemorrhagic induration.(Plate No.6).. A renal cyst, on microscopic examination was found to be filled with a pure mycelial culture of A.flavus series.

Plate No. 6

Haematuria cow, showing a greatly enlarged udder. It weighed about forty pounds. When the animal was autopsied and the udder sectioned, lesions similar to those of the bladder were present in profusion. There was no difficulty in finding the *Aspergillus* as the responsible agent in the process. This is a singular finding because the disease has hitherto been supposed to be only a bladder affection, (*Haematuria vesicalis*). There is reason to believe that when the udder is similarly affected, the infection is passed through the milk to suckling calves, and a number of such suspicious cases have come to notice.

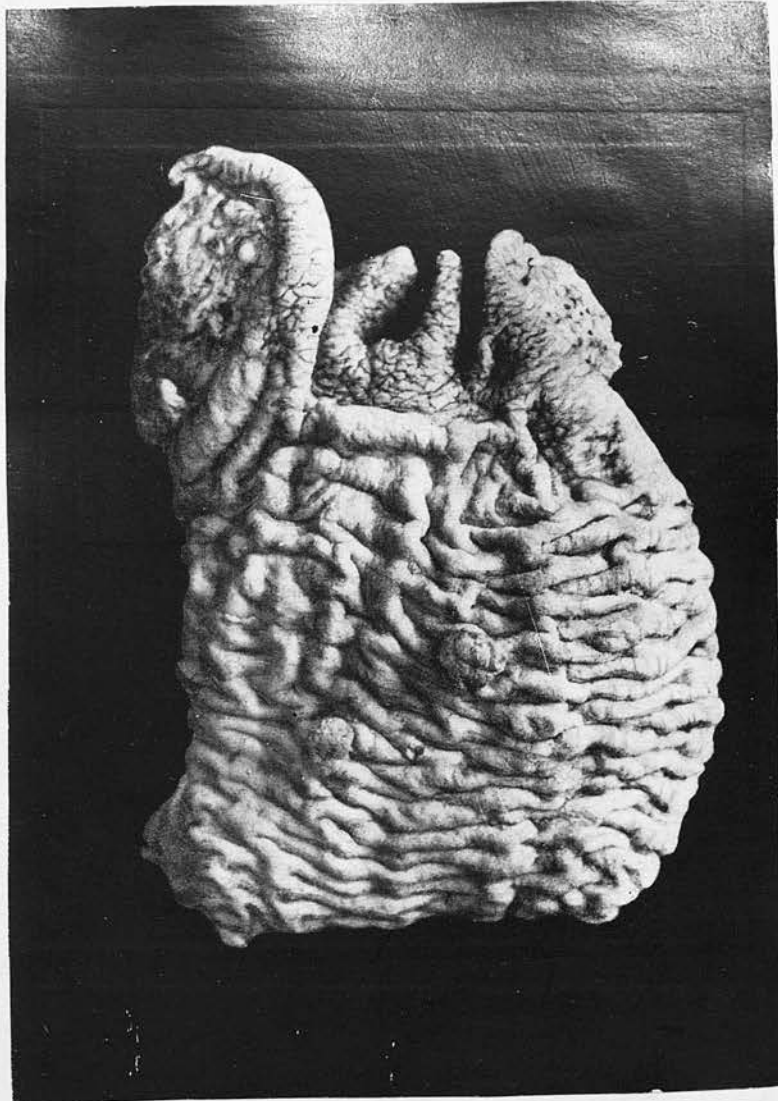
Plate No. 7

Fig.I. Two warty growths in the duodenum, a few inches from the pyloric end of the abomasum. Microscopically, sections showed resemblance to bladder-growths and the presence of rare parasites. From a natural case.

Fig.II. Portion of lung. Natural case of haematuria. Shows two localised aspergillar pseudotubercles. Proved microscopically.





Fig.I. In the course of microscopic examination of haematuria urine large globular bodies, similar to the above illustration, are found. Such bodies are also detected in diseased tissue from natural and experimentally transmitted haematuria cases. The significance of these bodies in the life history of the organism inside the animal body is not clear.cf.Plate 25,4(a).

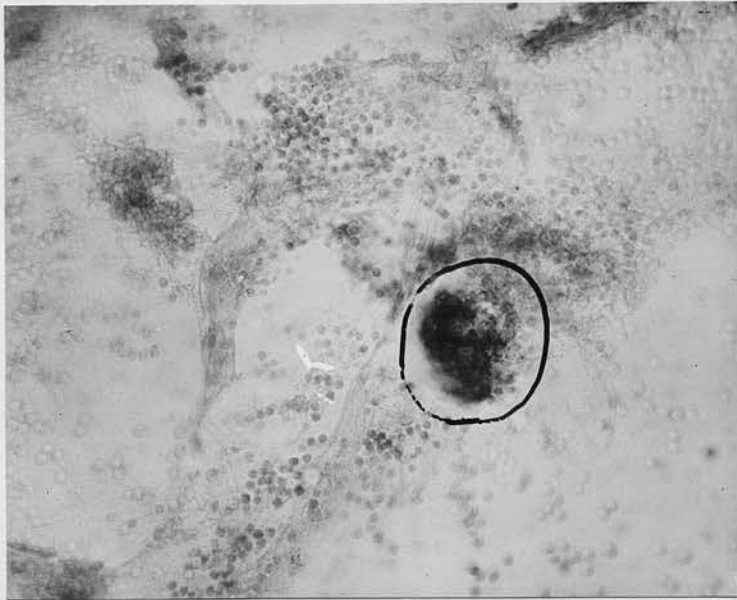


Fig.II. Smear from urinary sediment from a haematuria case in the Punjab, showed the occurrence of frequent perithecia. One of these perithecia is seen above in the centre of the field as a dark looking mass, due to its periphery being not shown up well under the stain used.

X 350.



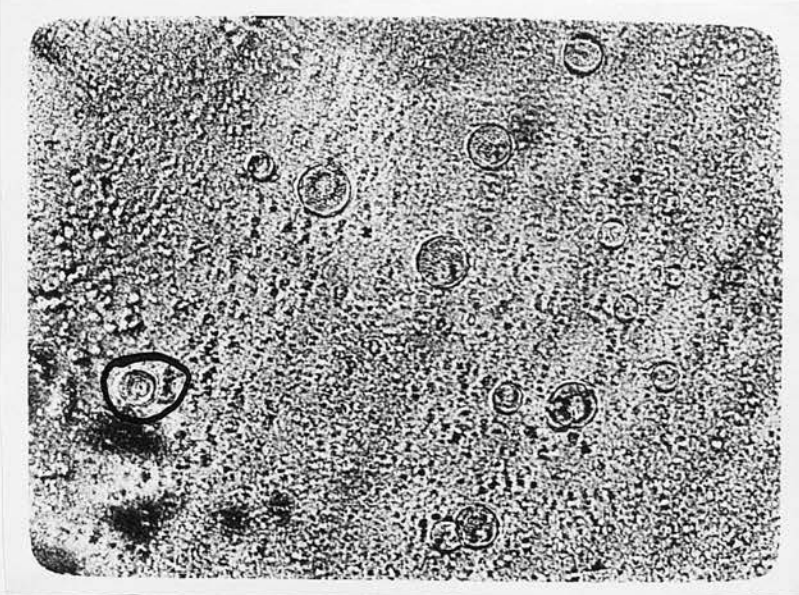
Plate No. 9

Fig.I. Centrifuged urinary deposit from natural case, treated with 10% caustic potash. Unstained. The body tissue cells have been broken down, leaving these peculiar rounded bodies of varying sizes, with a central ring. A small dot is noticeable in the centre of the inner ring. When these bodies were first detected, along with similar others in sections of the diseased bladder, it became necessary to carry out extensive cultural studies to determine their exact nature. These were suspected to be protozoan cysts, somewhat suggestive of Entamoeba histolytica. Reference may be made to Plate No.10.Fig.II, showing how these bodies were finally identified on artificial reproduction in culture. There is ample evidence to show that these large bodies were encountered by several previous workers, some of whom were inclined to interpret them as protozoa, but they failed to cultivate them.(see pages 62,63,64,66 ante) cf.Plate 24.

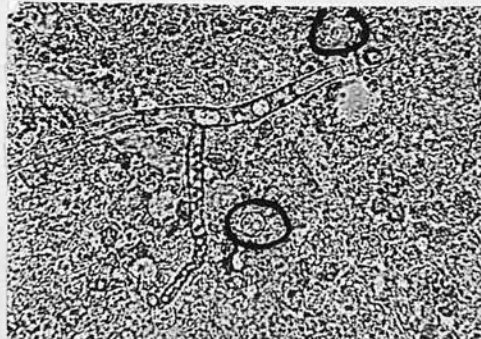


Fig.II. Scraping smear from the kidney. Natural case. Unstained and untreated. Lower magnification. Shows a number of the same bodies, though not so clear, and a branched hypha. They are chlamydospores, as far as can be judged. Plate 24.

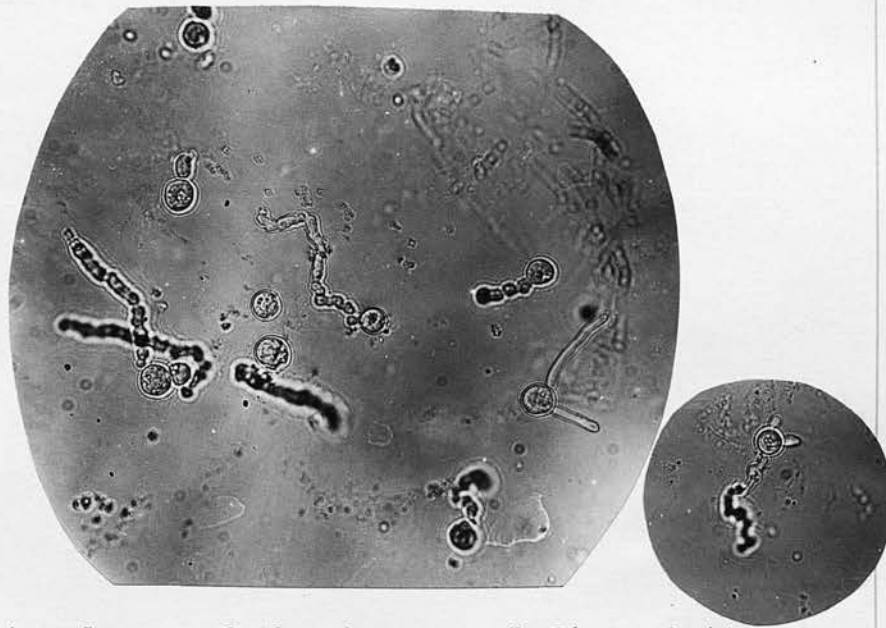
Plate No.10

Fig.I. A culture of the fungus. Bodies similar to these were repeatedly found in haematuria urine, by previous workers. When these bodies were first found to resist the action of caustic potash in urinary sediments or in bladder tissue smears, the present writer became suspicious that they represented certain parasites, possibly amoebic cysts. In one of these early preparations the finding of the production of hyphae as shown above, revealed their real nature.

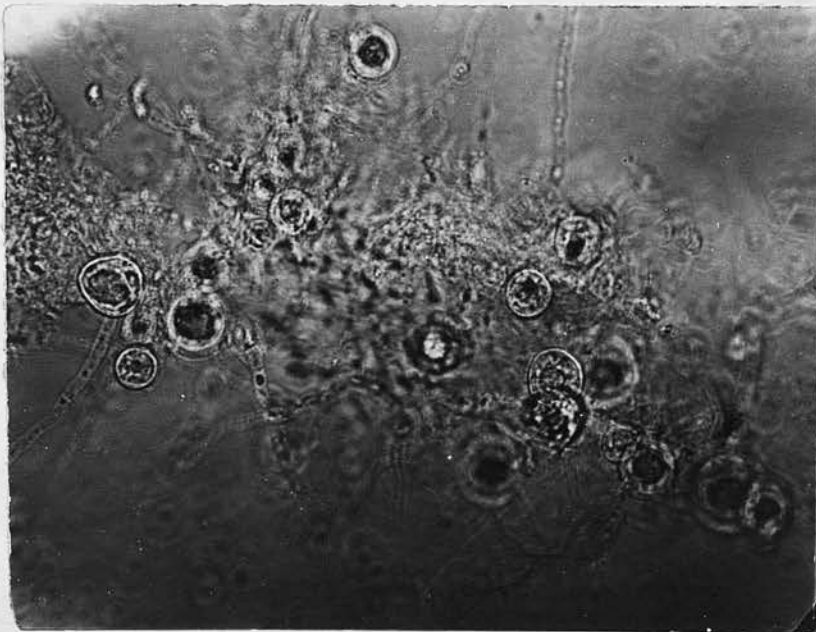


Fig.II. Same as above. The process was reversed. The bodies being produced on the mycelia. They were thus differentiated from body tissue cells, protozoa, and pus cells. The resemblance of these bodies to those already found in sections of the diseased tissue, is striking. Called 'degenerated epithelial cells' by others. Plate 23.

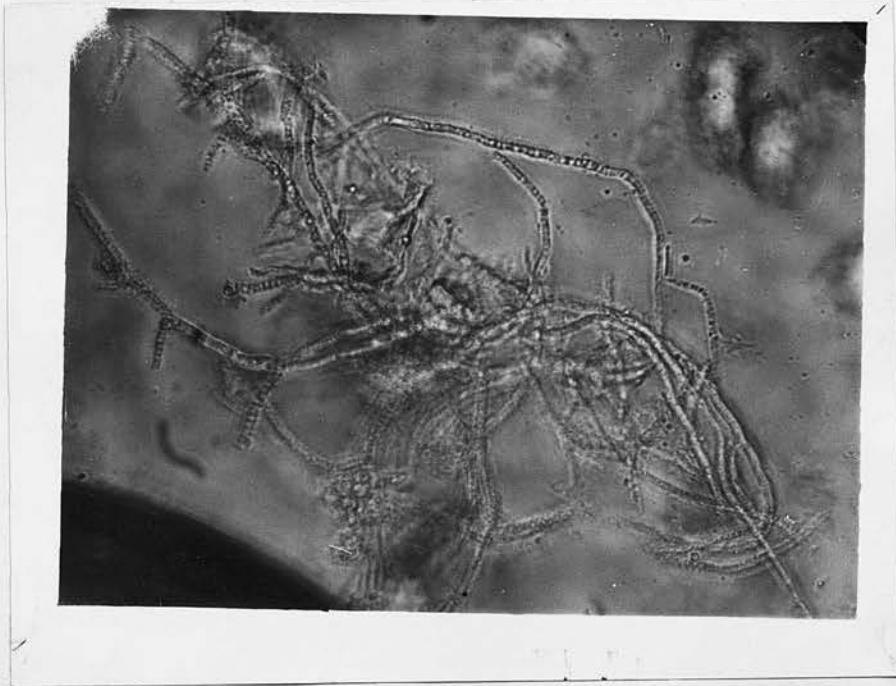


Fig.I. Scraping smear, from bladder. Natural case, when treated with either caustic potash, lactic acid, cotton blue or lactic-acid cotton blue reveals the presence of fungal network. The relations of the fungus to the different layers of the organ, particularly its distribution inside the diseased areas are clearly brought out. The character of branching, segmentation and hyphal granulation can be seen. This preparation was made from an Irish specimen.



Scraping smear. A pair of conidiophores, with stalk, parent hypha, a few sterigmata and commencing conidial production, as seen in scraping smear from the bladder. The size of the conidiophore is such that the foot cell, and vesicle, which should differentiate it from that of *Penicillium*, are not well-developed and can only be clearly seen occasionally and with persistence. Conidiophores of this type are invariably present in haematuria tissues from all the countries examined. The production of the same type of conidiophore by pure cultures of the *Aspergillus flavus* series has been repeatedly confirmed. The above smear was prepared from Irish material. X 400



(b) Histopathological Studies.

(i) Material and Methods.

Morbid materials from cases of the disease in Bengal had already been examined by the writer. On transfer to the Institute at Mukteswar in 1930, he took over the few clinical cases then living there, together with the existing collection of preserved pathological materials from previous cases. The earliest collected specimen was dated 1-6-22. It was found that abnormal urinary bladders, kidneys with greyish nodules, and occasionally lesions in the liver, duodenum, lungs and spleen, etc., encountered in the post-mortem room, had been included in the collection. The writer has since collected and preserved his own specimens. Materials were also received from other affected areas in India, either as a result of a special request or sent spontaneously. These have also been included in the present studies. Through the courtesy of the late Miyamoto, stained sections were received which were illustrative of the disease among cattle and buffaloes in Formosa. The departmental authorities in Australia and Canada were kind enough to supply preserved tissue from cases in their respective countries. An interesting specimen of an affected urinary bladder was secured



Fig.I. Diseased bladder. Natural case. Intensely stained H.E. Epithelium considerably proliferated, and drawn into crypts or folds. Submucosa shows increased numbers of dilated blood channels of various kinds in different stages of formation. A focus of dense cellular infiltration is also seen. Fungal elements were present in these lesions. Under low magnification.



Fig.II. Diseased bladder. Natural case. H.E.. Epithelium vestigial or practically lost. The nodular elevation on the inner surface of the bladder is due to the extremely dilated vascular structures, forming the centre of concentrated parasitic activity. Under low magnification.

recently from Ireland, from Professor Kearney at Dublin.

It may be mentioned here that the previously collected materials were mostly preserved in 10 per cent formalin, being moved after initial fixation to 70 per cent alcohol. Bouin's fluid and Schaudinn's fixative were employed wherever special requirements of the studies necessitated such a course. Latterly, Fleming's fixative has also been used with fresh material or with material passed through a preliminary formalin fixation. Of these fixatives Fleming's was found to be the best, and Bouin's fairly satisfactory, while formalin appeared to be the most unsuitable one to use. It was found in these studies that satisfactory results depended to a very great extent upon the correct fixation of the material to be examined, and the method of fixation adopted appeared to be important. Materials were also obtained from enzootic areas, by special request, in 50 per cent sterile glycerine, for direct microscopic examination and for cultural purposes.

Regarding the nature and choice of the materials available, it is to be noted that portions of the bladder and kidney were available from as many as ninety clinical cases of the disease, including material from other parts of the world, and in a number



Fig.I. Diseased bladder. Natural case. Low magnification. Stained H.E.. The epithelial layer replaced by 'alveolar nodes', really the 'parasitic nests', by the shedding of the epithelium and extreme dilation of mucosal and submucosal vessels. When the 'nodes' in the supervening layer give way and discharge the contents into the urine, the subjacent tissues are brought into immediate contact with the urine

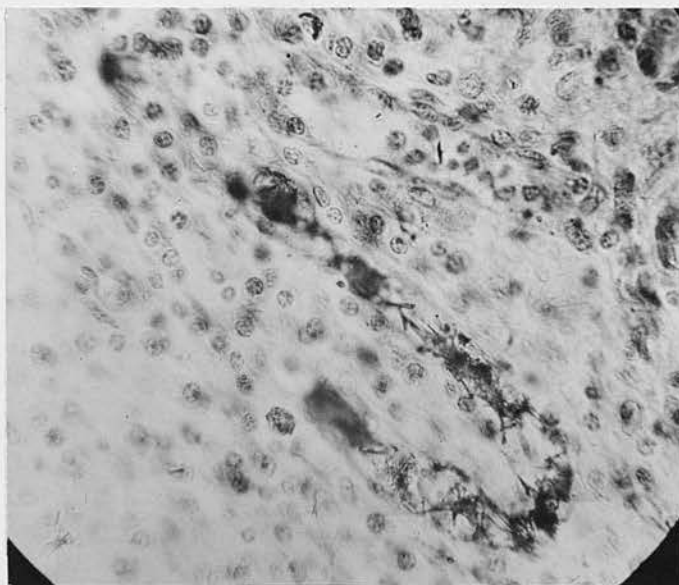


Fig.II. Section of diseased bladder. Natural case. Stained Feulgen and light green. Moderate magnification. A looped vessel is seen impacted throughout with luxuriant mould growth. The hyphal structures stand out more prominently than the free cellular structures. It is of interest to recall that Renon(1896) detected bladder lesions, and fungal elements in the urine of his experimental small animals, injected with cultures of Aspergillus fumigatus, and he ascribed this to the transport of the parasites through the venous blood.



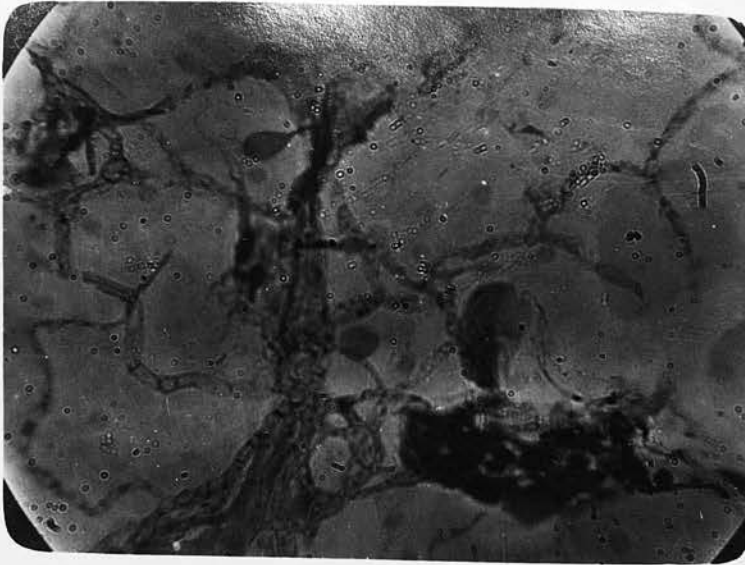


Fig.I. Section of urinary bladder. Natural case. In view of the unsatisfactory results usually obtained with Gram's method in the study of haematuria, various modifications of the technique were tried, including the prolongation of the time of staining to 24, 36 and 48 hours, and alterations in the process of destaining. The above field shows branching mycelial strands of the bladder disease under prolonged Gram staining. The results are not always uniform.

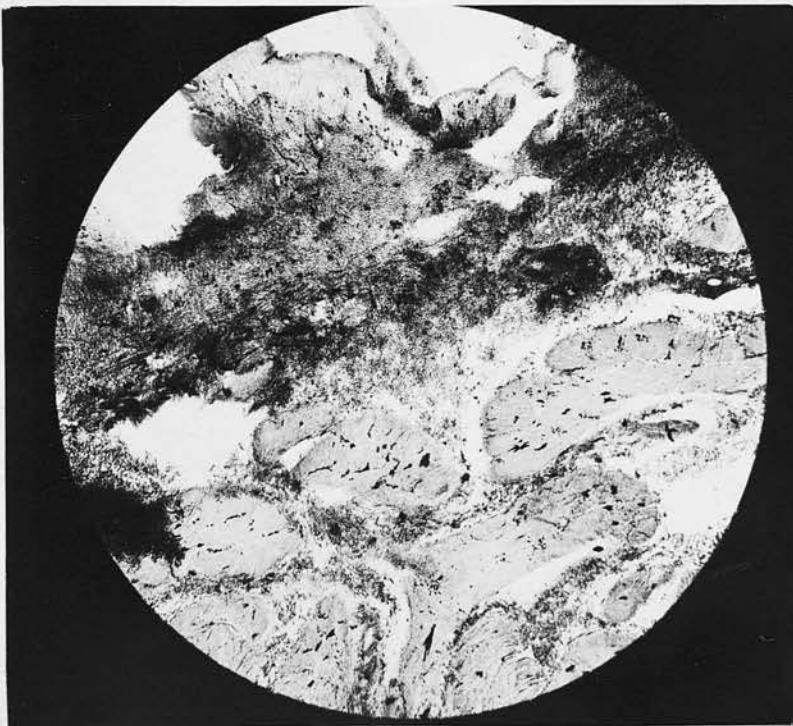


Fig.II. Section of a haematuria bladder. Stained by Feulgen and light green. Shows the general distribution of the fungus colonies as masses of dark deposits lying between tissue strands, either in filamentous form or in large or small irregular masses. This technique is capable of picking up the fungus colonies even in the midst of intensely haemorrhagic areas.

of cases certain other organs and tissues were also available. The fresh materials examined were mostly collected by the writer himself from clinical cases that either died or were destroyed for research purposes, in the field or at the headquarters at Mukteswar. Generally speaking, the materials in the majority of cases were collected because of some obvious abnormalities having been detected with the naked eye, though at the time of the collection no definite connection between the lesions and the disease entity - haematuria could perhaps be foreseen. From time to time, histological examination of other organs of affected cases was made, with a view to obtaining a general impression of the state of health, or of the degree of any abnormalities in different parts of the animal body that might be present. Instances thus occurred where the earlier negative material had to be looked up over and over again, in order to ascertain the validity of any new factor that emerged in the course of examination. Structures which first eluded detection were thus brought to light. The collection of pathological specimens involved in these studies has thus been very comprehensive and varied. The materials were subjected to repeated examinations from different angles over a period of about ten years, depending upon the

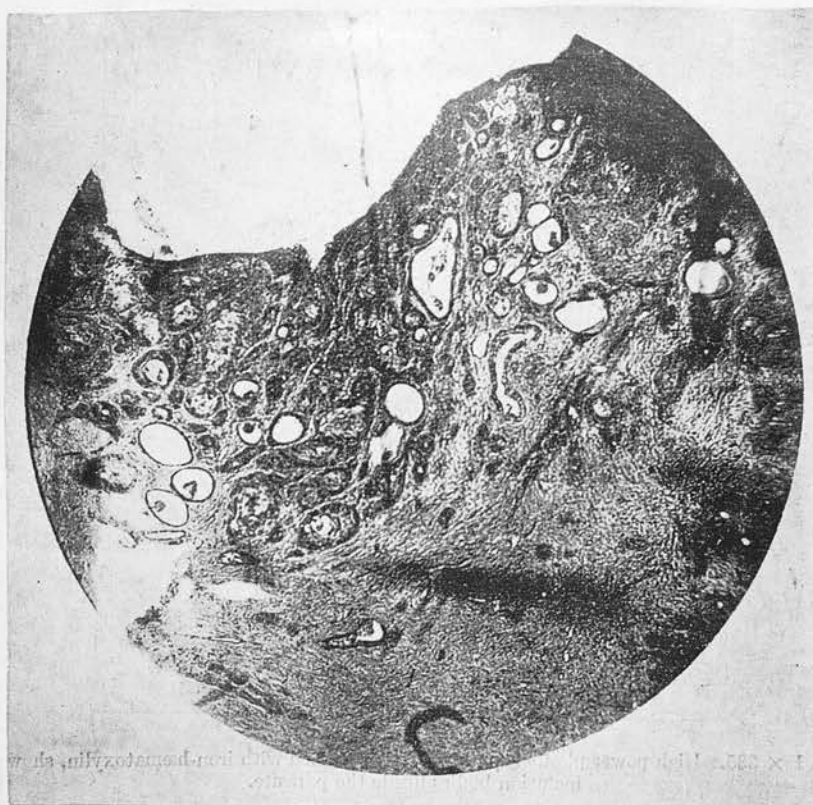
Plate No.16

Fig.I. Bladder. Natural case. H.E.. Shows growths highly suggestive of an adeno-carcinoma extending from the mucosa to considerable distance into the sub-mucosa.

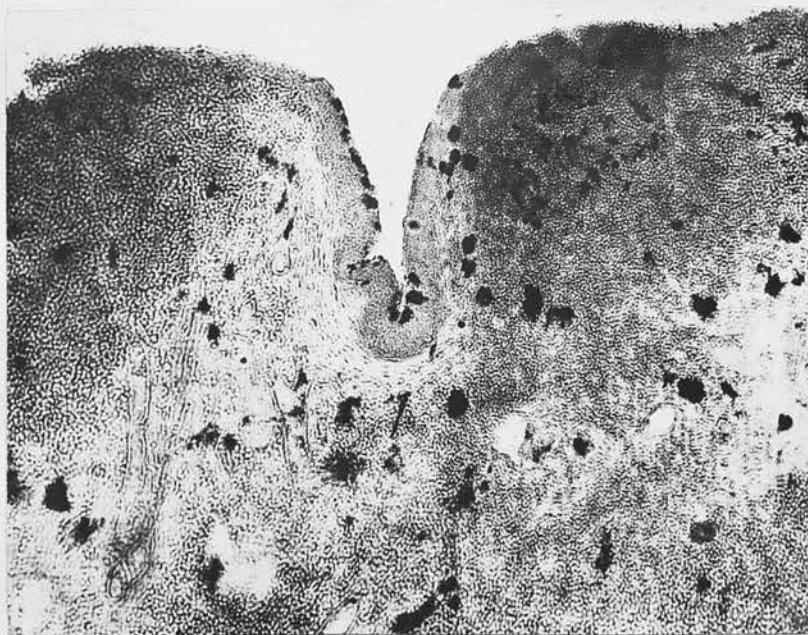


Fig.II. Section of diseased bladder, stained Feulgen and light green. The distribution of the black colonies, which represent fungus aggregations, is to be noted. Low magnification.



Plate No.17

Fig.I. Section of bladder. Natural case. H.E.. Shows infiltrating character of cell extension in the depth of the bladder wall.

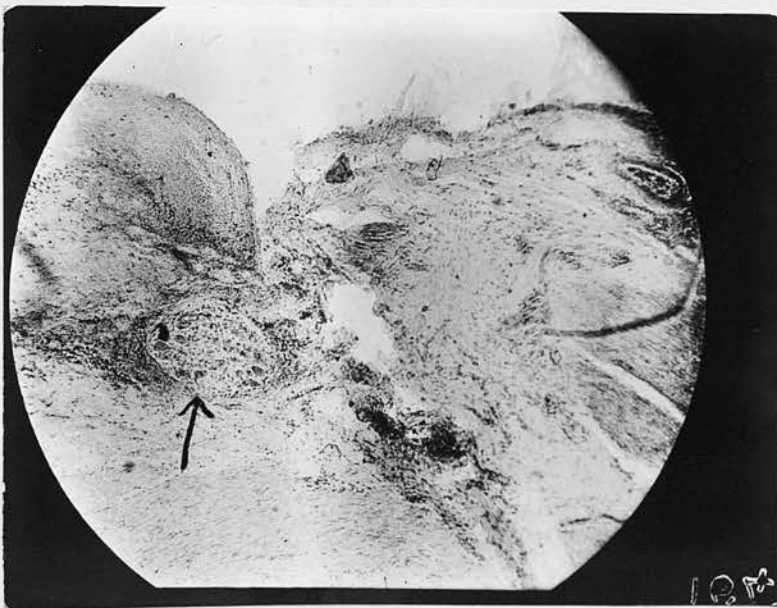


Fig.II. Section of bladder. From a natural case. H.E.. A peculiar colony, of ill-defined bodies, somewhat well bounded with considerable haemorrhage around it. The writer made the mistake of considering this as a colony of amoebic parasites, but has since found that it represented a nerve bundle.



particular aspects from which the problem appeared to be most tempting from time to time.

It seems advisable to mention here that the material from Australia was from an eight year old Shorthorn cow which had suffered from haematuria for three years. Material from an earlier stage of the disease, it was explained, could not be supplied. Like others, Australian workers were mystified to observe that an animal might pass very large quantities of blood, and even bleed to death, but that on post-mortem examination such an animal might not reveal lesions larger than a pin's head, and then only a few of these. The specimens from Canada represented an earlier stage of the disease. The veterinary authorities there mentioned in their covering letter that the animal from which the specimens had been taken, showed the first noticeable symptoms of haematuria on 15th October, 1934, and that it was destroyed on 19th November of the same year. That the materials from this case were collected after about a month from the starting of obvious haematuria, is of some interest and may be a helpful guide in the collection of suitable material. This Canadian material was found to be particularly interesting. (Plate 28)

The Formosa material, as already mentioned, comprised only a few stained sections, (the majority being

stained by the Heidenhain's Iron Haematoxylin method), and they confirmed the special findings of the present writer, already recorded with materials from elsewhere. It may be added here, that the materials studied by the previous workers consisted almost exclusively of urinary bladders, since the disease came to be regarded as more or less a pure bladder disease (Haematuria vesicalis) with practically no possibility of any other organ being involved. Abnormalities were, however, detected in other organs as well, but they were seldom studied, and then only by a very few workers. The co-existence of some of the common but unimportant lesions, like hydatid cysts and liver-fluke infestations, appears to have been responsible for creating this wrong notion.

#### Methods.

The usual histopathological methods were employed by previous workers, including staining methods for histological details, as well as the recognised specific stains for the demonstration of particular pathogens, like bacteria, protozoa, helminths, etc. Among the staining methods, Haematoxylin and eosin, Heidenhain method, Van Giesson stain, Gram's method, Leishman, Giemsa, Ziehl Neelsen methods were used. These, and some modifications of them, were tried by the successive workers upon this problem. A careful

search of the literature shows that with very few notable exceptions, data of significance or as pointing to a clue have not been recorded.

It is considered advisable to point out in parenthesis, that the general methods of bacteriology are applicable to the study of fungi only to a limited extent, contrary to the current belief. While, for instance, the bacterial stains may serve to a certain extent the general purposes of study upon a few mould-fungi in tissues, they are usually unsuitable as tending to obscure the details of fungal morphology. Moreover, the writer has repeatedly found that the ordinary histological methods of fixation, blocking and sectioning, produce considerable disturbance in the arrangement of the constituent structures of the fungus in the animal tissues. It would appear, therefore, that the mycological details of the organism invading the tissues are better studied in situ, without the fungal structures being disturbed from their natural relations. Lesser drastic treatments of the tissue should always be aimed at, with a view to obtaining a satisfactory preparation of the fungal structures, which is by no means an easy matter to achieve.

The Gram's method is recognised as a most useful method of dividing both bacteria and fungi into two

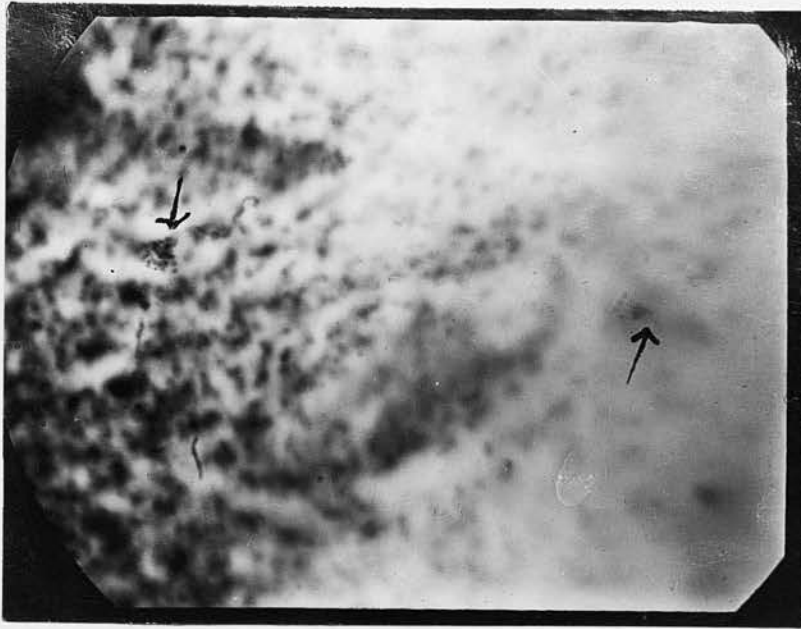


Fig.I. Section of the bladder from a natural case of the disease. Stained with H.E.. The field formed the site of active lesions, containing six conidiophores, of which two are in focus, and can be recognised.

X370.



Fig. II. Section of the bladder from a natural case, stained H.E., illustrating the distribution of the mycelial network. The darker portion of the field represents an acute haemorrhagic area.

X 350.



groups, depending upon whether alcohol or anilin xylol will decolourise them, following mordanting with Gram's iodine and staining with either Gentian violet, crystal violet or methyl violet. In this method, dilute carbol-fuchsin is used as a counter-stain to show up the destained gram-negative organisms. It is important to appreciate the scope of this reaction in relation to Gram-negative fungi, and particularly as a method of demonstrating the presence of Aspergilli in animal tissues. To quote Mallory and Wright (1924), "Gram-positive organisms are much more easily demonstrated in tissues than those which do not stain by Gram, because it is possible to stain them one colour and the nuclei of the cells another colour. In other words, it is possible to stain them so that they are differentiated from the tissue in which they lie, and hence stand out prominently. Gram-negative organisms have no differential staining; they take the same colour as the nuclei of the tissue. Moreover, although they stain easily, most of them do not stain deeply, and readily part with the colour they have taken up." Henrici (1914) carried out some experiments on Gram staining and states: "Moulds stain irregularly, isolated granules in the mycelia retaining the stain, while large areas do not stain at all." More specifically

with regard to the staining of *Aspergilli*, Lucet (1897) attempted the staining of *A. fumigatus* in the affected tissues. He records that when stained with methylene blue for microbes, only negative results were obtained, while with other methods certain small round bodies were seen, the nature of which was confirmed culturally. In the lung and kidneys no mycelium was demonstrable. He adds that other methods were equally unsatisfactory as they did not reveal any microbes in the animal tissues definitely known to be experimentally infected. More recently, Savage and Isa (1932), working upon aspergillosis of the poultry in America, state: "It was exceedingly difficult, however, to demonstrate the infecting agent. Dozens of staining methods were tried, but not more than a few fragments of mycelium could be recognised in any one section." Workers have also tried to reverse the Gram's phenomenon with a view to showing up the Gram-negative organisms while the Gram-positive ones would become destained. Among those who attempted it, Benians (1920) concluded: "The results obtained are not uniform and the degrees of differentiation are not sufficient." Investigating cases of abortion in cattle due to mycotic infection, Bendixen and Plum (1929) state <sup>(in German):</sup> / In all sections one could find many quite unstained hyphae. In several cases

it was quite impossible to stain the hyphae either with carbol methylene blue or with carm-alum or picric acid and indigo carmine. The hyphae were in all cases Gram-negative. Brown and Brenn (1931) attempted to modify the Gram's method by employing it as a differential method of showing up the Gram-negative organisms in tissue sections. Although the method was satisfactory for certain organisms, they found that an aspergillus could not be well stained in tissues.

With a view to obviating these difficulties, Lucet (1897) describes the method that gave him the best results: A fresh pseudo-tubercle (aspergillar) is isolated, then steeped in a solution of 20 per cent caustic potash till all the tissue elements are dissolved away, then washed in water. It is then cleared in alcohol-ether and finally stained by Gram-Weigert.

In the present studies, the routine methods already employed by previous workers were first tried. Besides, sections of haematuria bladder and other tissues were stained by a variety of methods not previously used by any one in these studies, e.g. modified Claudius method, Henrici's method (Jl. Med. Research, 1914, 30), Mallory's Eosin and Methylene blue (Jl. Med. Research, 1904), Mallory's Aniline

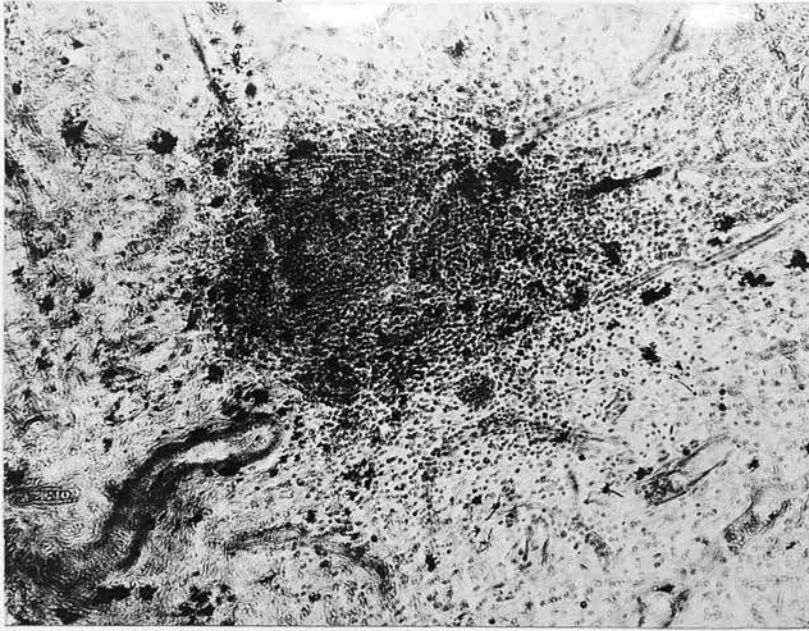


Fig.I. Section of the bladder. Natural case. Stained Feulgen and light green, showing cellular infiltration at the bifurcation of a blood vessel, as a reaction against the 'black deposits' representing colonies of the fungus.

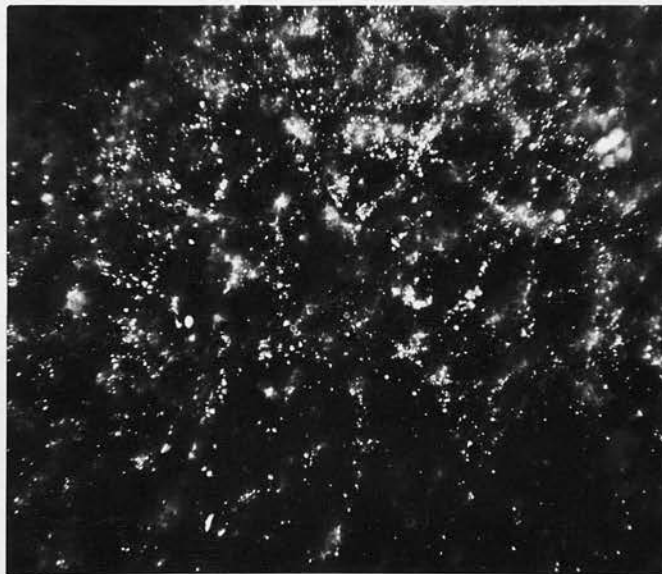


Fig.II. Section of urinary bladder. Natural case. H.E.. Taken under polarised light. Intensely haemorrhagic zone shows up the presence of doubly refractile bodies, borne on a branching network, which probably represent fungal elements and the chemical products elaborated by them.

X 220.



blue, Gram-Nicolle and modification, Gram-Weigert-Kuhne and modification (Pallin used them in Equine Epizootic lymphangitis), Murray's Osmic Acid method, carmine staining method (Am. Jl. Trop. Med., 8), Best's specific carmine staining method, and modifications of Gram's method by prolonging the staining. While these methods revealed the presence of mycelia and spores of fungus to varying extents in all the haematuria specimens studied, the best results were, however, secured by one or other of the following methods. (Often it was found that a combination of methods was better than any individual technique):--

(i) Careful examination of unstained sections and scraped shreds of diseased tissue, by the naked eye, followed by a microscopic examination.

(ii) Examination as above, after treatment variously with glycerine, caustic potash, lactic acid, and lactic acid-cotton blue. Lactic acid forms a suitable mounting medium of satisfactory refractive index, while cotton blue stains almost specifically fungal spores and hyphae. The last mentioned combination is a very useful method, but has the serious limitation that the preparations thus obtained cannot be kept permanently. Regarding the caustic potash to be used upon paraffin sections or shreds of infected material, it must be added that the strength

of 10-20 per cent is required. The strength usually used in plant pathology is much lower, varying from 1-3 per cent in most cases. On the addition of a drop or two of this detergent, a cover glass can be applied carefully, and the preparation examined under the microscope under a subdued light. A drop or two of glycerine may be run in if required. Rarely a stronger solution of caustic potash may be required.

(iii) After mounting in fluids of different refractive index, glycerine, xylol, Gurr's mounting medium, alcohol, water, thick and <sup>(xylol-chloroform-)</sup> thin/Canada balsam. The fungal mycelia being delicate, extremely hyalin and transparent, the variation in the mounting medium may considerably influence the picture presented.

(iv) Staining of tissue smears with iodine, osmic acid, picro-aniline, cotton blue.

(v) Staining of paraffin sections with different dyes after fixation by different methods, artefacts being guarded against by the use of different methods successively, e.g. Bouin's fixative, Fleming's, Schaudinn's, formalin fixation, followed by Fleming's or by osmic acid alone. Osmic acid is picked up and retained by the fungal network to a greater extent than by the animal tissues. (Plate 33, Fig. II).

(vi) The application of Feulgen's reaction, followed by light green counterstaining. <sup>and supplementary haematoxylin treatment</sup> Fungal

Plate No.20

Fig.I. Section of bladder. Natural case. H.E.. Shows two blood vessels close to the serous coat in a state of irritation and sclerosis..

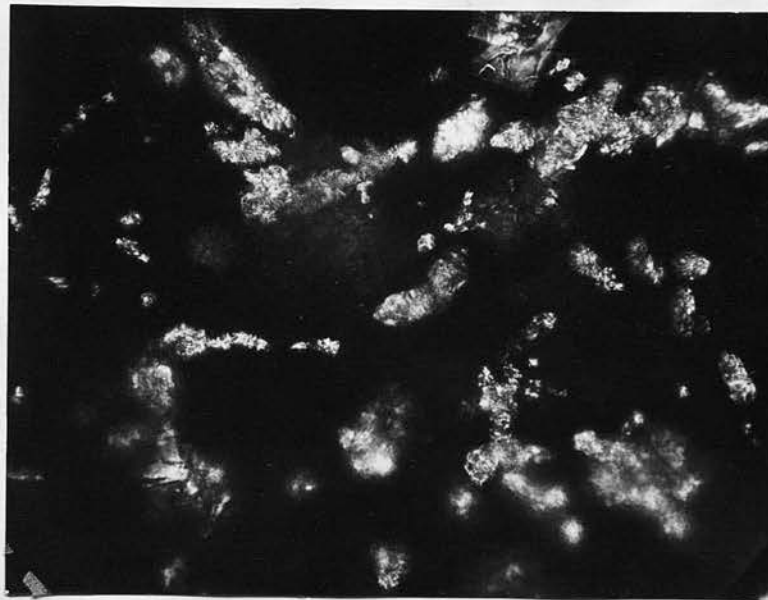


Fig.II. Section of diseased bladder. Natural case. Showing colonies of the fungus encrusted with salts. Note the connections of the colonies along their length. Polarised light. X 500.

colonies and conidial heads are readily picked up, and their general distribution over the whole section can be estimated.

(vii) Staining of sections, after treatment with 0.3% chromic acid, 1% caustic potash, 2% osmic acid, or 1 in 1000 solution of potassium permanganate, with different histological or bacterial stains. The stainability of the organisms is increased by these chemical agents.

(viii) The use of polarised light. Depending upon the thickness of conidial heads, the amount of salt incrustations, particularly of Calcium Oxalate crystals and pigments produced by them, the globular heads stand out brilliantly illuminated against the dark background of diseased bladder tissue, when a section is put on the stage of polarising microscope. The presence of the doubly refractive aspergillar heads, and crystals in the bladder tissue, can be revealed by utilising their power of rotating the plane of polarisation (anisotropism). (Plates 40, 65, 19 & 20).



(b) Pathological Histology of the Lesions.

Urinary bladder.

From the earlier discussion under symptomatology, it will be clear that it is not usually possible to detect a case of bovine haematuria at its very commencement, with a view to collecting specimens for studying the earliest stage of the pathological process. The passage of blood in the urine in sufficient concentration to attract notice for the first time means that the pathological process has managed to involve the mucous and submucous linings, rather than that it represents the earliest stage of the disease. The young and old lesions are however present in most bladders, and these can be utilised for histo-pathological studies.

The disease would appear to be predominantly an affection of the submucous layer, inflammatory exudation into which can nearly always be detected. The blood vessels supplying the mucous and submucous layers, whether they be minute capillaries or large sized vessels, are greatly dilated. The capillary vessels are often ruptured and degrees of extravasation take place. The whole bladder is intensely congested, while in some cases the submucous areas are provided with large numbers of well-defined

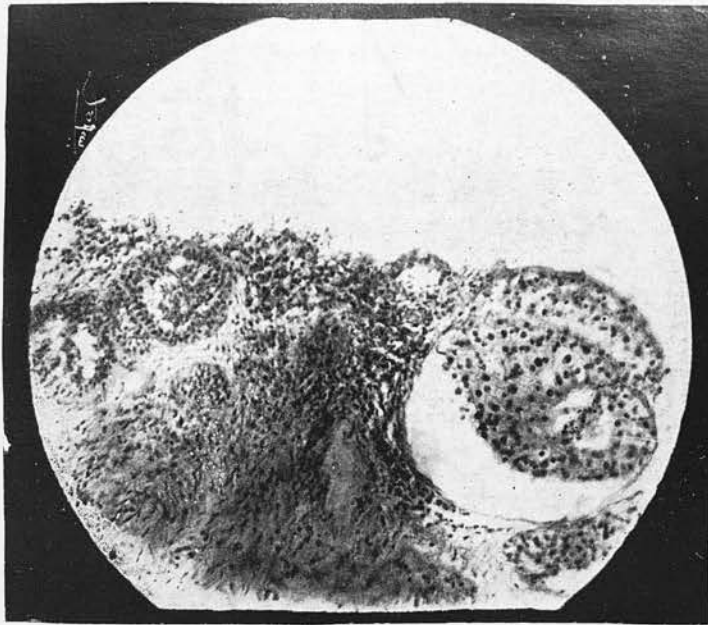
Plate No.21

Fig.I. Section of bladder. Natural case. H.E.. Several solid nests of epithelial nodes, disposed in the form of rounded adenomatous structure without any evident lumen, but with degeneration a thinning and shedding of the enclosed tissue takes place. As degeneration progresses certain nucleated bodies become more prominent and numerous. Presumably these nodes are centres of parasitic activity inside blood vessels. The largest node is nearing the process of shedding.

X 143.

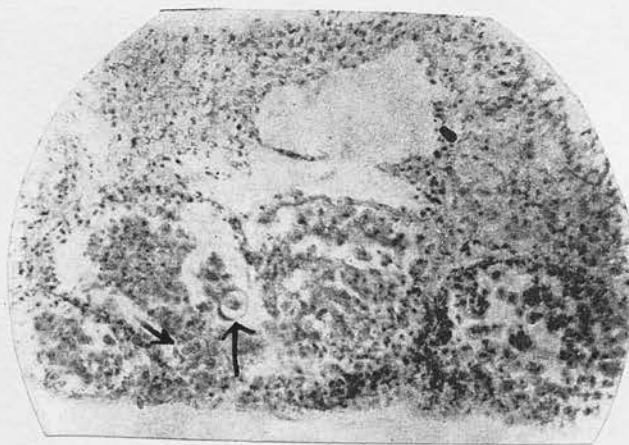


Fig.II. Nodes as above with a prominent large parasitic cyst. Presumably bodies of this kind were the structures recorded by several previous workers including Arnold in Germany, Goetz in Switzerland, Case in Hawaii Islands, Roger in France and Scharer in Antiquoa.

X 128

pools of blood. These changes are generally more pronounced in some parts than in others. In some cases, however, the whole of the bladder wall is intensely infiltrated with blood and its pigments. The vascular endothelium is damaged to varying extents, showing a tendency towards hyperplasia or shedding. In the perivascular regions a pushing apart of the surrounding connective tissues is quite frequently seen, resulting perhaps from the exudation of serum and the migration of cells. Sometimes an oedematous infiltration is very pronounced, and is easily discernible immediately below the muscularis mucosa. The blood vessels present degrees of a distinct tendency towards endarteritis obliterans, and the changes may be very marked.

As a result of the inflammatory exudation, the epithelial lining of the bladder is deformed in patches being oedematous, swollen and elevated, the inflammatory products separating the mucosa from the thickened submucous layer. The nucleus of some of the cells is distorted. A lining of delicate fungus filaments may be detected in both the mucous and the submucous layers, but they stand out more clearly in the latter. The extravasated blood and exudation are partly absorbed or removed. When the amount of this is more than can conveniently be disposed of,

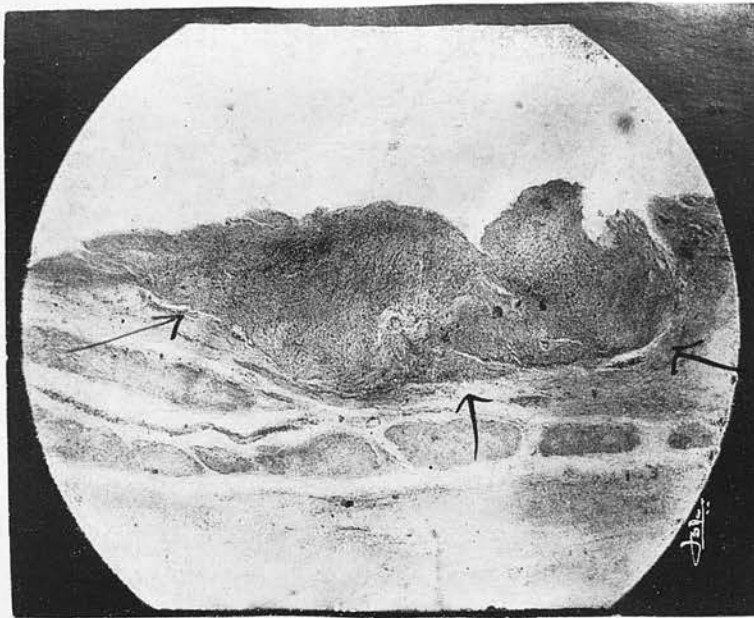
Plate No.22

Fig.I. Section of bladder. Natural case. H.E..  
Showing a rather deep ulcer, almost reaching to the  
muscular layer. X 244.

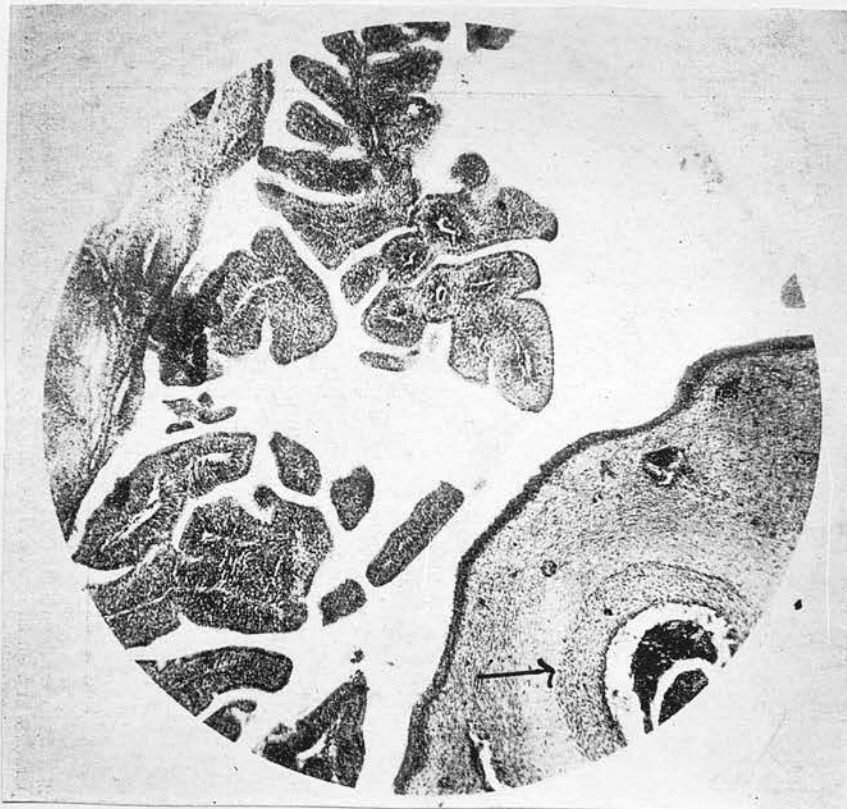


Fig.II. As above. Shows warty papillomatous processes provided with a central core of blood vessel. Note the thickening of the wall of the vessel at the right-hand side. Low magnification.



degenerative changes in the overlying epithelium take place. There may be degrees of necrosis leading to the formation of ulcers by sloughing. The immediate irritants are thus removed with the slough and haemorrhage, and organisation of the broken epithelium commences if no further irritation is continued. Clots are formed on the raw surfaces and local capillary thrombosis may take place. Deposits of old clots may be found adherent to the lesions. In the more insidious cases of haematuria, the post-mortem examination of the bladder in the active stage of haematuria fails to show any marked bladder lesions. A number of subepithelial vessels are found to open widely into the bladder cavity, the blood being thus discharged directly into the urine. When these vessels are closed, or a clot formation takes place, the extravasation blood ceases with haematuria disappearing for the time being. The seat of the closed vessel or attempted cicatrisation remains a weakened spot. Further progress of the recuperative mechanism may be undermined by the animal straining its bladder wall, together with the intervention of a fresh instalment of parasitic activity, presumably due to an excessive and rapid production<sup>of conidia.</sup> Even if the obliterating clots or thrombi were to persist and the lesions were to be completely resolved, fresh lesions

would continue to appear elsewhere with the exhibition of haematuria. The disease thus obtains a foothold and with the greater extension of the vegetative mycelia, it tends to become chronic. The result is that diverse types of lesions which characterise the disease, manifest themselves. The lining epithelium of the bladder may still appear to be intact in the major part, but small ulcers or even ulcerated patches may be present here and there. Generally these areas of ulceration are very superficial and would seldom appear to extend below the submucosal layer. In well established cases, however, deep ulceration and necrotic tissue may be encountered. <sup>(Plate 22, Fig. I)</sup> Secondary infections appear to play some part in these extensive lesions. Immediately under the epithelium and extending only a relatively short distance into the deeper tissues are the blood cavities and channels of various shapes and sizes, <sup>(Plate 13, Figs. I & II)</sup> mentioned earlier. The connective tissue stroma which supports these extremely vascular or "haemorrhoidal" areas, appears to have undergone some amount of hyperplastic change. These blood cavities have been called "angiomatous" or "haemangiomatous" by previous workers. Apart from the several scattered points where the denuded areas of the mucous membrane are in the process of cicatrization, one notices a proliferation and thickening

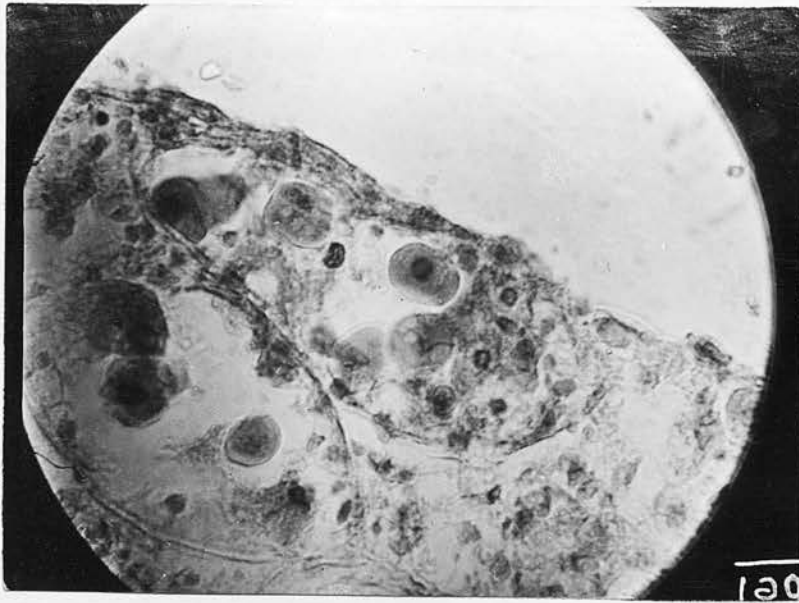
Plate No.23

Fig.I. Section of bladder. Natural case. Intensely stained by Gram. Colony of large sized parasites in the superficial lesion of the bladder. They presented the typical ringed character with a central dot of an Entamoeba nucleus, and as opposed to bloated endothelial cells, they stained comparatively well. When such preparations were shown to a well-known medical protozoologist, he gave the opinion that if the tissue was from a human being he would have no hesitation to state that these peculiar bodies were Entamoebae. cf. Plate 9 and 10.



Fig.II. Section of bladder. Natural case. H.E.. Shows a colony of parasites, and a double-contoured cyst, localised in an extremely attenuated epithelial node on the point of bursting. X 216.

of the epithelial lining. Obviously the series of changes through which the affected mucosa passes must depend upon the degree and frequency of the irritation (presumably the production of conidia and their budding), as also upon the chronicity of the case. In such an area of the mucosa, where the traumatic or irritative influences have not directly operated, or where they have not been unusually severe at one point or points, as by the simultaneous production of a huge crop of conidia or other reproductive bodies, the intact epithelium has to adapt itself to the mild irritations to which it is being continually subjected from time to time, due to the inroads of the gradually advancing, ramified hyphae. The bladder tissues attempt to adjust themselves to changed circumstances by the production of branching cauliflower-like tassels of soft papillomatous growths. (Plate 22, Fig. II) The character of the epithelium of the bladder is maintained over these growths, and branches of blood vessels are carried into each prolongation. A few pyriform mucoid cysts are formed, since the escape of the secretion of the mucous glands of the bladder is obstructed.

In the continuity of the bladder epithelium, along with the proliferated cells, (Plate 21) one often notices several solid nests of epithelium which are generally disposed in the form of rounded adenomatous cysts,



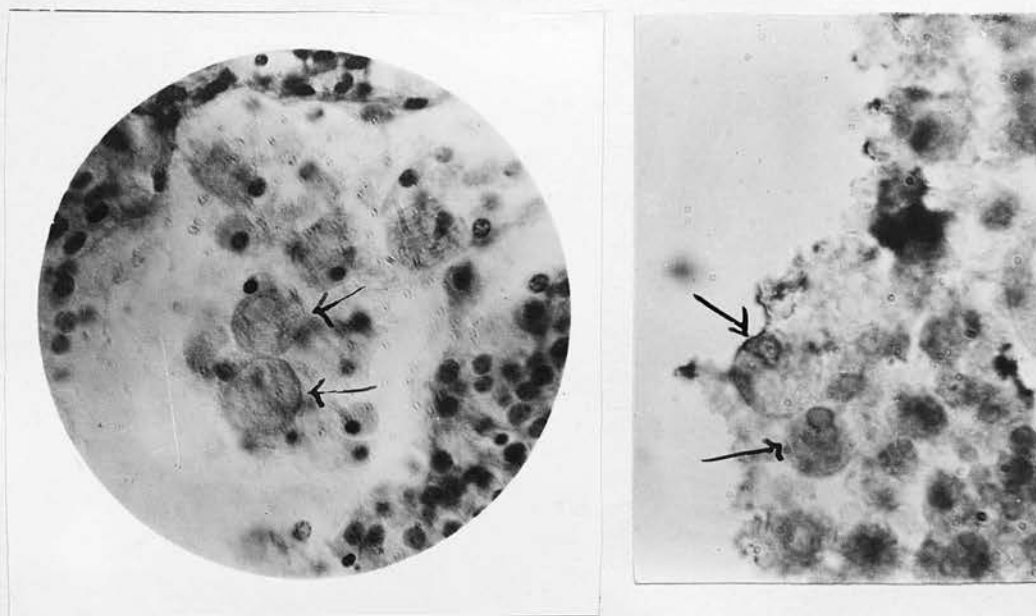
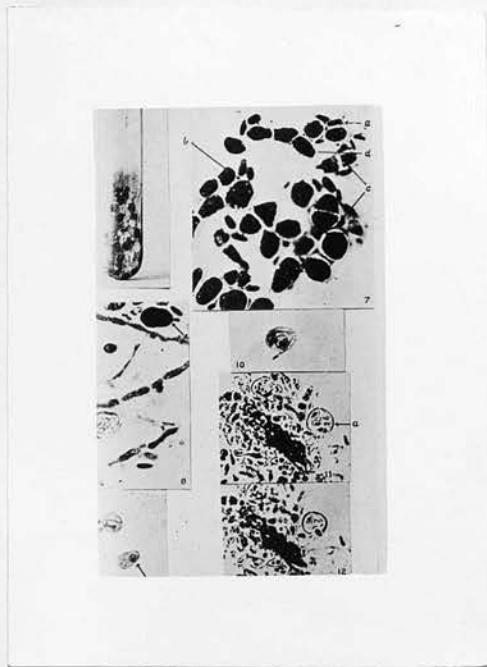
Plate No.24

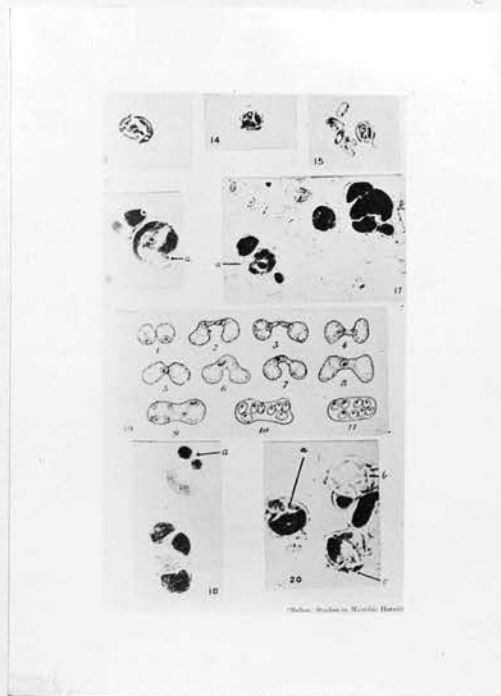
Fig.I.& II. Bladder sections from natural cases. Stained H.E.. Show peculiar cells. Those on the left are vacuolated but little other inner differentiation can be made out. Previous workers have seen them and were inclined to believe that they were degenerated epithelial cell. Some have even considered them to be parasitic. In old cultures of fungus these highly vacuolated involution forms have been produced in the present investigation and are therefore etiologically connected to the disease. Of the parasites on the right the character of the nucleus may be noted. (1) X 186. (2) X 450.



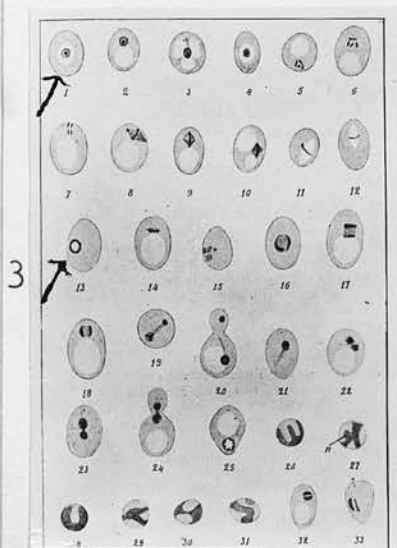
Fig.III. The centre of the field shows clearly the characteristic appearance of the nucleus and the central dot of chromatin, which though highly suggestive of an amoebic organism, is actually the nuclear pattern of the fungus parasite of haematuria. cf. Plate 9.



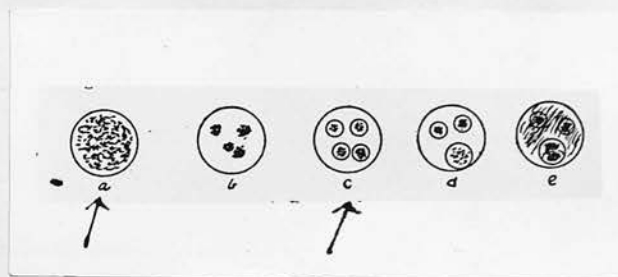
1



2



3



4

The above illustrations have been reproduced from Mellon's article on "Studies in Microbic Heredity", and from another publication. From the cytological standpoint, and for the striking resemblance in the nuclear pattern of these fungal organisms to that of the haematuria parasite, the above illustrations are highly interesting and may be of assistance in the future in furthering studies upon obscure aspects of Aspergillar cytology. Note the resemblance of 3 (1), 3 (13) and 4 (c) of this plate to stages of *Entamoeba Histolytica*. Also cf. 4(a) above with Plate 8, Fig. 1.

but apparently without any central lumen. Various stages of development and degeneration of these epithelial nodes are evident. As these nodes enlarge, their central zone shows some thinning, while a few prominent nucleated bodies are clearly seen. <sup>Plate 21, Fig. 1</sup> With further enlargement of these nodes, the nucleated bodies show increased structural differentiation.

Some definitely double-capsuled bodies are also <sup>Plates 21 & 23, Fig. 2</sup> found, and the evidence of phagocytosis or parasitic encystment is manifested. The collection of several layered epithelium in nodes appears to be more frequent than the normally existent mucus-secreting glands of the bladder. The available evidence seems to indicate that a number of these nodes may originate from the bladder epithelium, though the exact mechanism is rather difficult to picture. A number of such nodes are also found to be present in the depth of the bladder. There need be little doubt that these originate from blood vessels in which the endothelial layer has undergone considerable proliferation.

There is nothing to indicate that such is not really the mechanism of the formation of nodes in the epithelial lining itself. It is conceivable that the papilliform processes of the mucosa as they grow, may close and form tubular adenomatous structures. (Plate 26, Fig. I.) Degenerative changes in the nodes extend from the

centre towards the periphery, and in the advanced stages all the epithelial cells may be destroyed, (Plate 23, Fig. 2) giving place to a collection of an increasing number of the parasitic incitants (which are perhaps the conidia, vesicles and germ tubes). The enclosing membrane of the node then bursts and the contents are liberated into the urine.

Another abnormality found to be invariably present (by all workers) in the bladder sections consists of scattered minute collections of cellular infiltration, (Pl. 13, 19, 27, Fig. I) generally situated immediately beneath the epithelium and also around the ulcerated or haemorrhagic lesions. Some workers have interpreted these as sarcomatous. The cells are generally of the mononuclear type, consisting of what appear to be lymphocytes, young fibroblasts, endothelial cells with an admixture of so-called plasma cells. In sections subjected to a preliminary treatment with weak caustic potash solution before the application of haematoxylin-eosin and Gram methods, and also with other methods including the Feulgen reaction, the nature of the irritant in these foci has been determined to be germ tubes, conidia, branching mycelia. Even the actual conidial head has been discovered. (Plate 18, Fig. I) A capillary vessel is sometimes discernible in the centre of the foci of cellular reaction. (Plate 19, Fig. I).



- Plate No.26

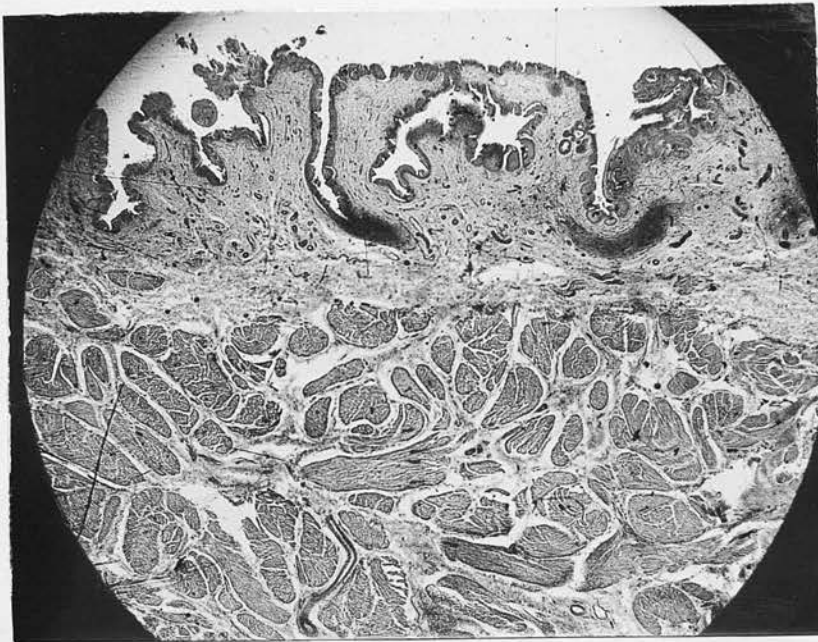


Fig.I. Section of bladder. Natural case. Low magnification. Shows tendency of papilliform processes to come close together and coalesce forming adenomatous structure.

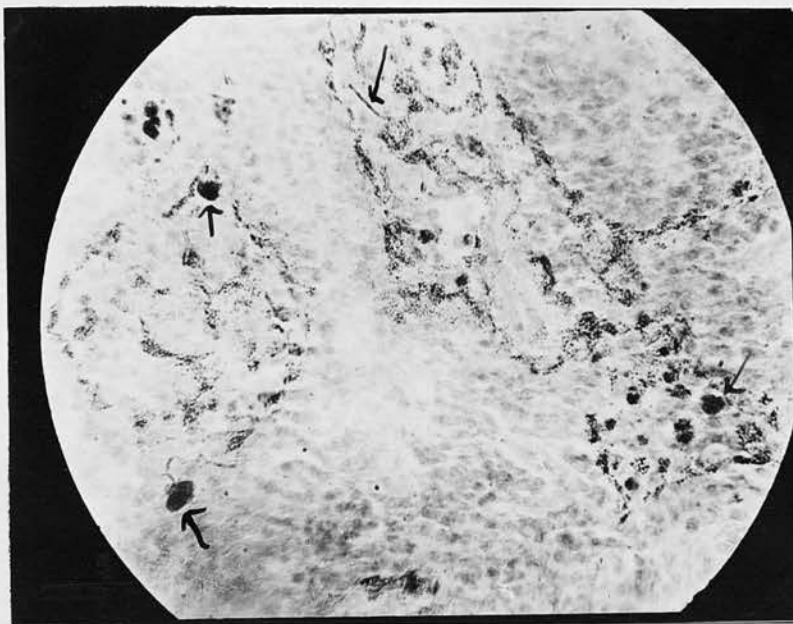


Fig.II. Section of bladder. Natural case. Shows strings of highly granular mycelia in the tissue with a few rounded parasitic bodies. X 300.

The fundamental lesions of the disease are, however, formed in the small and large vessels which show definite reactions within and around themselves, irrespective of their situation in the thickness of the bladder wall. The submucosal vessels appear to be the first to exhibit the lesions of the disease, and, as already mentioned, the majority of the vessels are more or less dilated, and this dilation is accentuated in some places more than in others. (Pl. 13, Fig. II). Further, the endothelium of affected vessels shows proliferation, either at points or all over the lining membrane. Similarly, the desquamation of the endothelium may take place at points or be general. The presence of the parasitic thallus in its characteristic forms around and inside the lumen and wall of the affected vessels, is the most striking picture to be seen. (Pl. 14, Fig. II) The blood vessels of the area become enormously dilated, their walls give way in places, haemorrhages result and blood oozes out of the mucous surface through the minute breaches between its cells. Thrombus is also formed. Some of the vessels are actually obliterated, while others are partially sclerosed. In most old-standing cases the blood vessels at all depths of the bladder wall show an extreme degree of proliferation of the endothelium, and appear as collections of epithelial cells only,

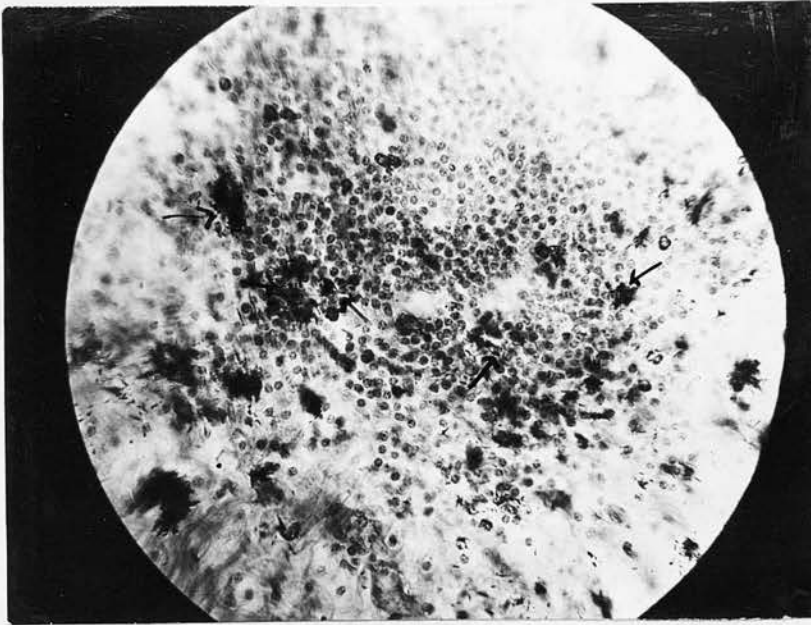
Plate No.27

Fig.I. A focus of cellular infiltration. From a section of diseased bladder. This lesion has been recorded in the bladder by practically every worker, though not from any other organ. In the present studies this lesion has been found in several internal organs including the liver, lung, kidney, spleen. The dark masses represent colonies of the fungus, acting as the irritant inciting the cellular response from the host. Stained by Feulgen and light green.

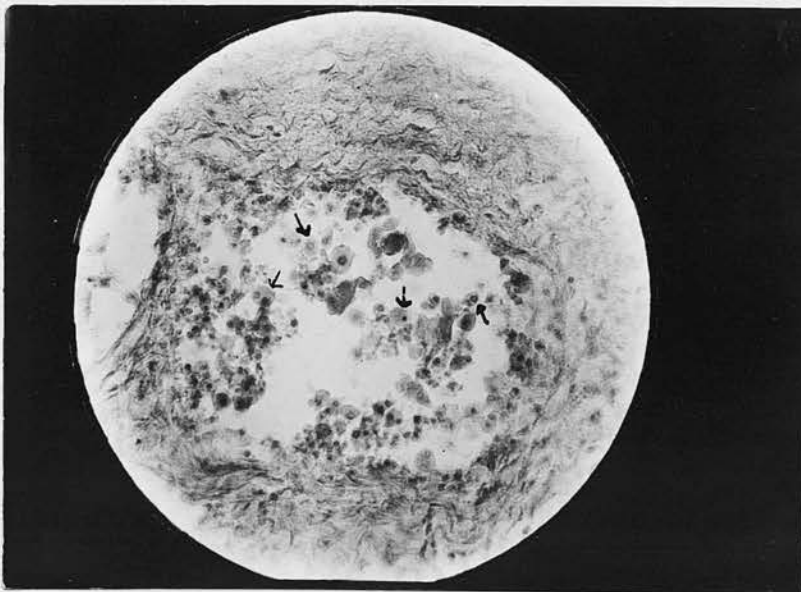


Fig.II. Section of bladder. Natural case. A common lesion is on view. Note the character of the nucleus and the cytoplasm and the extremely variable sizes of the congregated bodies. Parasitic activity is intense and the difficulty of differentiating parasites from tissue cells can be appreciated, but from potato-kidney sections studied the identification can be made.X.490.

bearing little or no resemblance to the original blood vessels. In some cases a definitely infiltrative type of cellular extension is manifested over parts of the organ, and the tendency to simulate malignancy is unmistakable. <sup>(Pl. 16 & 17, Fig. I)</sup> The so-called "birds' nests bodies" of epithelioma are practically never to be seen. It is doubtful if real metastasis of malignancy into other organs has yet been found, though a number of workers appear to be definite about it. It must be admitted that in rare instances the bladder changes are highly suspicious of adenocarcinomata, <sup>(Plate 16, Fig. I)</sup> and the Australian workers report the finding of carcinomatous change in the liver in haematuria cases. The writer has found similar changes in the udder of a cow.

Now with regard to the distribution and appearance of the parasitic agent, <sup>(Pl. 16, Fig. II, Pl. 19, Fig. I & II)</sup> it may first be repeated that they are found in their active forms in the areas of progressive haemorrhage and marked pathogenic action, such as in the mucous and submucous layers. One interesting point observed is that while the animal tissue cells lose their staining affinities as a result of degenerative processes, the fungal elements, particularly in their capsuled uninucleated stages, show a pronounced stainability of their nuclei. The fungus mycelia and even spores



may be found a considerable distance beyond the highly haemorrhagic zones, for instance, along the fibrous septa between the muscle bundles, and even between the individual muscle strands. Inside the congested and often dilated blood vessels, or in the haemorrhagic zones, comparatively large and dark stained aggregations of parasitic bodies are frequently seen, but sometimes it may be difficult to find them. Some of the large bodies appear to represent incompletely developed vesicles, which have failed to become conidial heads.

In quite a number of well-established cases, the muscular layer and the subserosa are heavily infected. In the muscle bundles, individual strands or groups of them may be found to be abruptly discontinuous in their length, or abnormally separated from their supporting strands, though in parts the musculature may be apparently healthy. These muscular lesions, on examination, reveal considerable mycelial activity, and when examined under caustic potash treatment, some sprout mycelia may also be revealed. (Plate 41, Fig. I & II).

In order to appreciate the structural arrangement of the fungus as it occurs in the tissues, it is advisable to recollect its appearance in artificial cultures. It is known that in cultures of most

Plate No.28

Perithecium measures 103.2 x 74.3microns.



Fig.I. Section of diseased bladder. Natural case. Stained H.E.. Submucosa shows actively degenerating lesion with a central small cell reaction. On examination this lesion showed mycelia, conidia and even typical Aspergillar heads. The epithelial layer shows mycelial strands penetrating through cellular spaces, and a fully developed perithecium suspended into the bladder cavity with a conidial head showing at the left hand side. Perithecia and conidial heads are occasionally found inside the bladder tissue at all depths of the organ. Sent in formalin from Canada. X 220.



Fig.II. As above. Higher magnification. Showing more than two conidial heads in the mucosa, the connecting mycelial network bears a perithecium practically on the mucosal surface. The network was seen ramifying luxuriantly into the depth of the organ. X 500.

multicellular fungi, the mycelial thallus is of two kinds, which can be differentiated as the basic or fixed vegetative portion, <sup>(Pl. 18, Fig. I & II)</sup> and the aerial reproductive portion. The former maintains an intimate contact with the substrate by burrowing into it to different depths and forming a more or less closely interwoven network, digesting and absorbing nutriment from its environment. The reproductive portion extends practically vertically into the air to a higher level obtaining a more liberal oxygen supply, and producing and discharging the reproductive elements, the "spores". It is this character of reproduction and vegetation which forms the basis of the botanical identification and classification. (Again the chronic and recurrent features of haematuria can be accounted for on the behaviour of the two parts of the thallus.) The chemistry of the organism appears to be conditioned by its physiology, and the staining reactions vary accordingly. Though under the available methods of study the organisation of a fungus gives the impression of a simple constitution, there is reason to believe that it must be as equally elaborately organised, to serve its biological functions, as the animal tissues.

In actual tissue sections, suitably treated or stained to show up the fungus, two types of mycelia

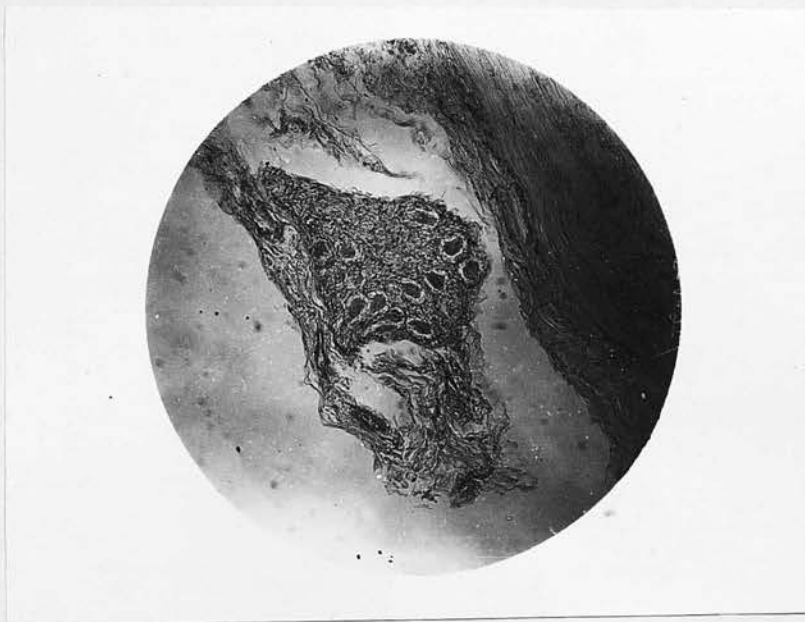


Fig.I. Section of the bladder. Natural case. Stained H.E.. Shows a nerve bundle near the serous coat of the organ, the individual nerve cells having undergone marked changes, presumably as a result of the disease process operating in haematuria.



Fig.II. Two nerve bundles in another section of diseased bladder. Stained H.E.. The nerve cells in one bundle have disappeared and those in the other show commencing degeneration. In Obici's (1898) studies on Aspergillosis, nerve cells were found to be changed into shapeless heaps of protoplasm and granules.



can also be recognised, analogous to the thallus described above in connection with artificial cultures. It is known that the differentiation of a fungus mycelium from such tissue elements as connective tissue strands or fibrin filaments, depends generally upon the characters of its branching, septation, distribution and relations with the neighbouring structures. The detection of a Grampositive fungus, or even of a Gramnegative organism if and when the latter assumes Grampositive properties, while lying in the centre of the disease or degeneration process, is relatively simple. In the disease under study, where the organism happens to be Gramnegative, and the mycelium growing in the midst of the animal tissue assumes a very fine and delicate appearance, its segmentation in most places may not be readily made out. (Plate 28) Other facts, like the presence of attached spores or refringent granules, may then assist diagnosis. Experience shows that the vegetative mycelium lies intertwined with the denser tissue elements, at times merging almost imperceptibly into the supporting stroma. Thus is the fungus somewhat obstructed, mechanically and optically, from the view. To try to find a sharp line of demarcation between the fungus and the animal tissues may sometimes become difficult, necessitating the use of special methods. It may be

stated in a general way that the mycelium may be differentiated by its failure to stain with Weigert's fibrin method, being Gramnegative, failing to give the reaction for elastic tissue, for iron, or for connective tissue by the Van Gieson method. The fungus is extremely hyaline and somewhat waxy in texture. Irrespective of whether the fungus in the tissue happens to be above or under the other in its relative situation, selective staining is not at all easy. The reason is that anything that has the possibilities of staining or decolourising the fungus, appears to have a similar intensity of effect on the histological elements of the body as well. As compared with the fixed vegetative mycelium referred to above, what appear to be parts of the reproductive mycelium, however, are found to be free and somewhat dangling. During the making of stained preparations, these may be dislodged to a very slightly different optical level on the section. For this reason and because the reproductive mycelium tends to become more stainfast than the vegetative portion, they are liable to be more readily recognised. It must be admitted that to some workers this may raise a difficulty of interpreting the picture presented by the relatively free reproductive mycelium. At first sight these fungal elements might be thought to be

extraneous organisms, growing on the surface of the sections as a result of the lack of technical care. This suspicion is dissipated as soon as the fungal strands are more closely examined and traced to their connections. (Plates 18 & 28) It then becomes amply clear that the dangling sort of hyphae are intimately connected to the vegetative mycelium in the ground tissue of the organ. It must be recognised that the liability of dislodging the mycelial "felt" is greatest in places where the diseased tissues (Plate 28, Fig. I) have been extensively drawn upon by the invading organism for its nutrition. This is well illustrated in the areas containing amounts of loose connective tissue strands, e.g. in the spaces between muscles, and around blood vessels. The degenerated tissues, moreover, seem to lose the power of holding the fungus in situ.

Experience has shown that there is, however, another important fungal element which is even more liable to be dislodged, viz. the conidia. If one adds some water to a culture tube, or to a petri culture, as carefully as possible, one can readily study the effects of surface tension on the conidia. The conidia are borne on the conidiophore so lightly that the slightest disturbance of the incoming water, or even of the air, disperses them. These organisms are commonly known as "powdery moulds", and if an old

petri culture is just tilted, considerable amounts of the "powder" run down to the lower edge of the plate. If paraffin sections of an *Aspergillus*-infected tissue are similarly tested under the microscope, each time they are put into grades of xylol, alcohol or water, or treated to the various reagents and stains, very illuminating information can be obtained regarding the speed and the extent of the actual dislodgment of the fungus elements, which must be guarded against in the usual processes involved in staining.

The fungus organism is not only highly pleomorphic but also polymorphic, being capable of assuming different forms depending upon the immediate environmental conditions in the tissues, the oxygen tension, the reaction, etc. It is difficult, and sometimes even impossible, to form an idea as to which stage of the fungus is represented by some of the forms encountered in the animal tissue. To decide whether some of those do not represent stages of some unimportant secondary organism, yeasts or other fungi, has required prolonged studies, particularly in cultures under varied conditions. To establish the identity of all the forms found in the tissues, it was found necessary to seek the aid of experimental studies of the morphological features and stainability of the different stages of the organism when grown in



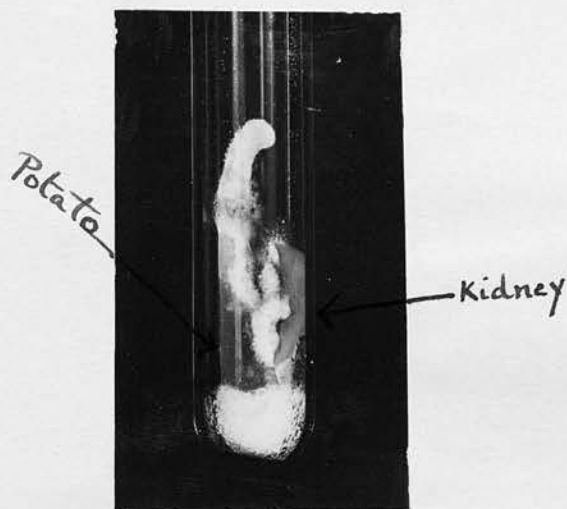
Plate No. 30

Fig.I. Culture tube. A portion of kidney from an experimentally infected rabbit, collected aseptically, inoculated on potato slant (left). Luxuriant growth at the junction. Sections were cut and stained, and some remarkable findings were made with regard to stages and characters of the organism, which were hitherto elusive. This method was found to be very useful in diagnosis of suspicious material.

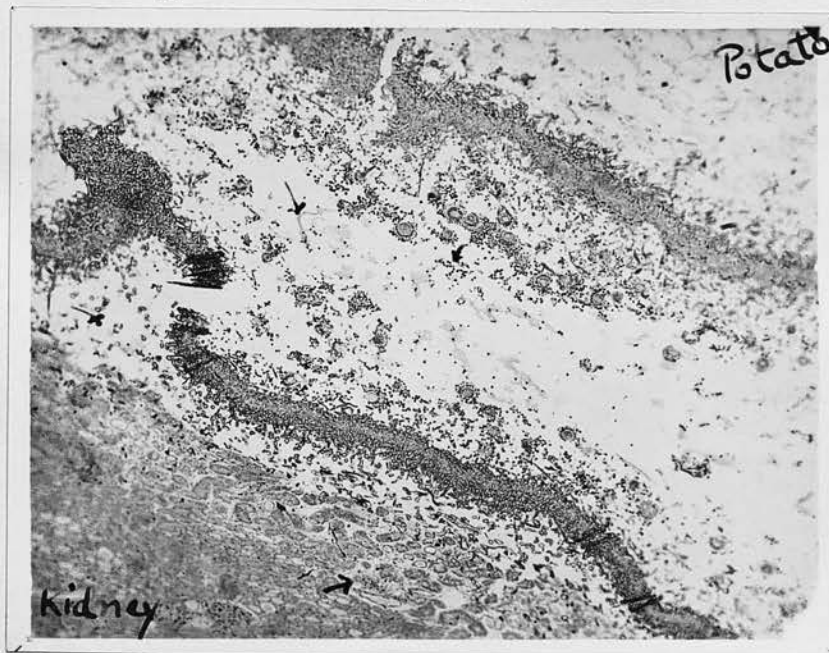


Fig.II. Section of above. Low magnification. H.E.. Note conidial heads and aerial growth at the junction bounded by two thick columns of intertwined mycelial ropes. The variation of the nature of fungal structures produced in the different parts of the intervening space between the potato (top right hand), and kidney (bottom left hand corner) is striking. The fungus assumes peculiar features inside the tissue.

X 60.

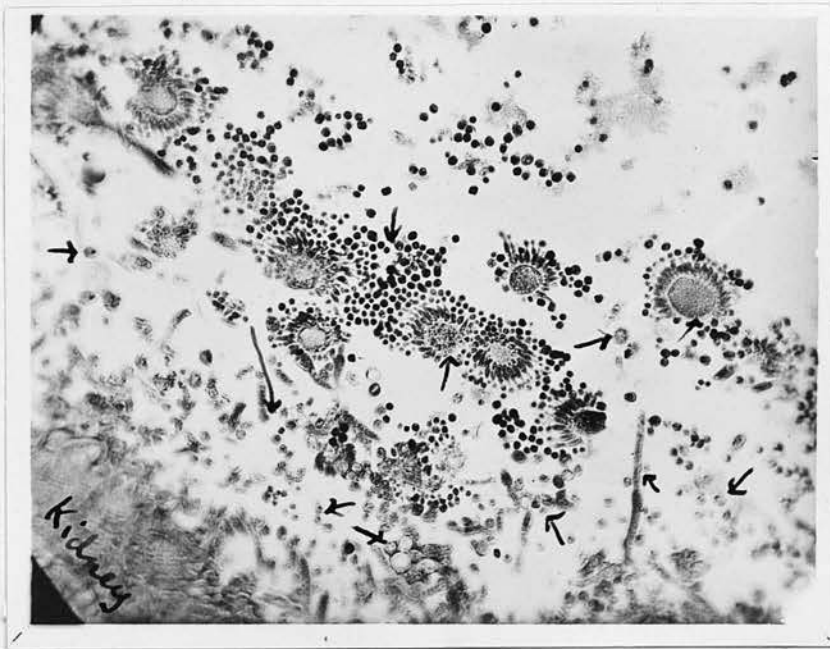
Plate No.31

Fig.I. 'Heads' and aerial growth at the junction of infected kidney sown on potato slant. Same as Plate No.30 Fig.II., but under higher magnification. Note the conidia, brightly stained away from the tissue, with those nearer the tissue being comparatively stain-resisting. The morphological details of the conidia, the variation in the relative size of the nucleus to that of the cytoplasm in different stages of both small and large conidia, and similar interesting details of the vesicle, sterigmata, and hyphae have been clearly brought out. The information obtained from these studies has been very illuminating, and explains satisfactorily the reasons why the identification or recognition of these structures in haematuria tissues has been so baffling. The difference in the staining reaction of the organisms in different stages, exhibited due to proximity or otherwise of animal tissue may be partly ascribed to the degree of oxygenation, but perhaps the existence of animal nutriment is also implicated in the production of chemical changes governing staining. As one examines the deeper parts of the affected tissue, the failure to stain becomes more and more accentuated. X 240.

pure culture on either vegetable (potato) or animal matter (healthy bladder tissue). A clear insight into the nature of the structures encountered, and information regarding the stages in the life history represented by each form was thus obtained by careful and prolonged microscopic studies of stained sections prepared from the healthy bladder tissue, or from sterile potato medium by cultivating the pure fungus strain upon them. These studies proved very helpful in enabling the interpretation of what was so difficult at first.

It was found that the shape and morphology of the organisms depended very much on the exact developmental stage in which the parasite happened to be, and upon the quality and quantity of the nutrition available to it. The process of the development of the forms encountered in the tissues appears to conform to that in cultures. As a result of studying sections of a piece of potato "seeded" directly with a piece of infected kidney, collected aseptically from an experimental animal succumbing to an artificial infection, (vide Plate 30, Fig.I&II), it was ascertained definitely that certain undifferentiated forms (Plate 31) were actually developed from the fungus./ In the actively proliferating state the fungal cells are generally small in size resembling lymphocytes. The

active adults may show a tendency to vacuolation, and some of them may be highly vacuolated. <sup>(Plate 24, Fig. I)</sup> The old and much vacuolated forms are perhaps the involution forms. These forms have been reproduced in pure cultures. (Plate 10, Fig. II). Some of the fungus elements, both in the mycelial stage and in the uninucleated stage, show a marked eosinophile tendency. Certain stages of the fungus are represented by rounded shapes, and by sizes varying within certain limits. These bodies were found in the pathological material, but in culture the sizes were inclined to vary, rendering the task of identification troublesome. Some of these rounded bodies or granules are extremely minute and may represent stored food material, but the possibility that the others are spores of some kind cannot be lost sight of. The suspicion that these represent some kind of an early developmental stage has come to the mind repeatedly. The fact that these minute bodies preponderate in animals succumbing to experimental infection with pure cultures of the fungus, and are also seen in the naturally affected tissues, suggests the process of "endosporulation" described by pathologists as occurring in animal tissues invaded by certain fungal organisms. Granules may also be formed by the breaking down of the blood or of nuclear substance of cells, but these minute



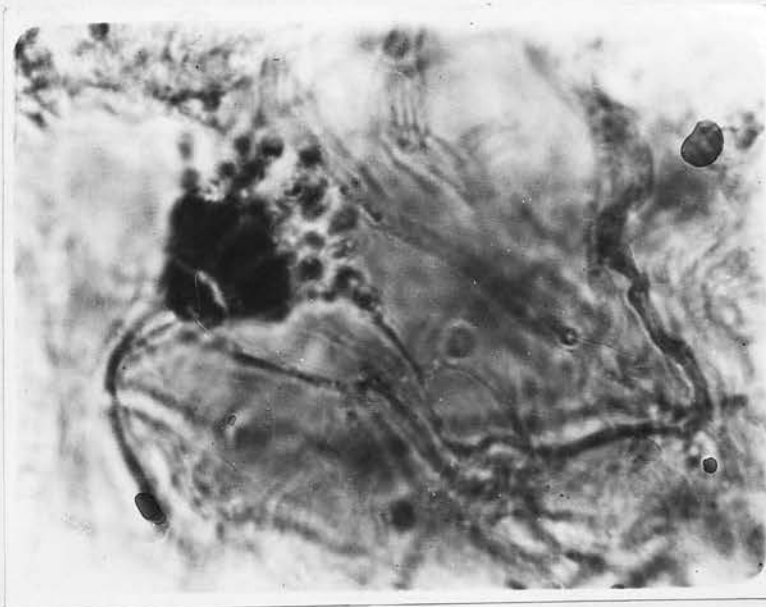


Fig.I. Section of diseased bladder, stained H.E., showing mycelial network and also conidial head. Note the relation of the vesicle, sterigmata and the radiating chains of conidia, and also the stalk.



Fig.II. A conidial head in the bladder tissue of a natural case, under high magnification, showing the morphology of the conidia. Note the empty looking area at the base, representing the vesicle.



Fig.III. Another conidiophore with its stalk, from a bladder section. A clump of loose conidia is seen above the conidial head.

bodies appear to be different. In some places the branching fungal strands bear appreciable numbers of uninucleate spores, probably chlamydospores. (Plate 33, Fig. I & II). In other parts, the mycelia are seen to be transformed to resistant bodies, like chains of cylindrical oidia-like cells distributed in the course of the hyphal tube. The conidia are comparatively readily recognisable in the tissue lesions. They are approximately of the same size as the red blood corpuscles and, like them, happen to be globular or oval and mildly eosinophilic in character. This fact explains how they have been missed by previous workers. When the conidial capsule is matured, it is inclined to retain the stains to an appreciable extent, particularly of haematoxylin and of Gram. It may be added that tissue sections also reveal what appear to be hollow hyphal tubes and abandoned membranous walls, resembling those found in old cultures.

The thallus consists of coarse hyphae with reduced refractility, or of fine fibrils dotted with refringent chlamydospores along their length. The tip of the germ tube is usually more refractile than the rest of the hyphae. When any part of the fungus thallus arrives by extension or by transport to a fresh favourable substratum, the trophic stage is started. It may be explained that the conidia are

Plate No. 33

Fig.I. View from section of diseased urinary bladder. Natural case. Shows mycelial network in a tissue space, provided with the dark stained 'spores', probably chlamydospores. Note that the spores are solidly stained, without any obvious demarcation of nucleus and cytoplasm. In cultures similar spores were produced. X 140.



Fig.II. As above. Another field from a separate case. Shows fewer spores but a considerable production of broad, what may be called shredded, mycelial strands. These are selectively picked up by Osmic acid, and Mallory's modified connective tissue stain used for showing up fungi in tissue. X 140.

apparently readily transported throughout the animal system, but the risk of a knot of mycelia being prevented from doing so, due to arrest in its passage towards the urinary bladder, is certainly considerable. Conidia, though typical in shape, appear to vary considerably in size and staining characters, but uniformly within certain limits. The conidia are generally seen lying singly, but two or three of them may be found attached together, either in one line, or at different levels or angles. The outer wall of the spore is sometimes shown up by special stains (e.g. stained blue in the Ziehl-Neelsen method or by Claudius' method). (Plate 36, Fig.II).

In a favourable situation the germination of the conidia or of the oidia-like segments takes place by a swelling of their body and extrusion of the germ tube. The germ tube may continue to grow when the conditions are particularly favourable. Otherwise the process of its elongation may suddenly cease, simulating a budded yeast cell. Contrary to the yeast, however, the potential property of extension of the germ tube is still retained, though for the time being, somewhat under check.

In the tissue the fungus appears to have several methods of propagation. This depends partly upon the stage of the fungus "seeded", whether in the





Fig.I. Atypical conidial heads with chains, from a section of bladder from a natural case. Compare with Plate No.61, Fig.II.

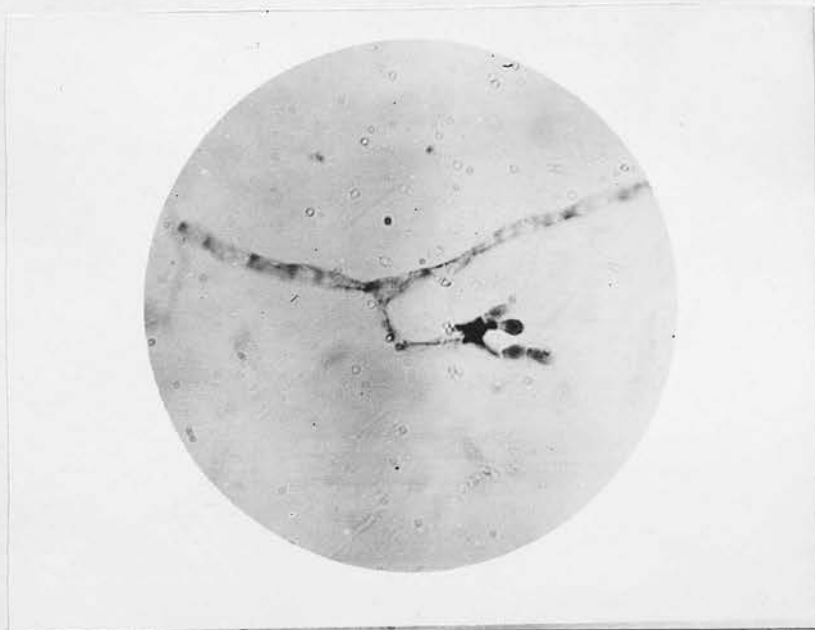


Fig.II. Atypical conidial chains growing at one point of the hypha. From section of diseased bladder. It will be of interest to note that (Mosseray, 1934, Plate III. Fig.13), identical forms have been encountered in other *Aspergilli*.

trophic stage, i.e., young and vigorously growing, or in the old and exhausted forms. The character of propagation appears to depend also on the environmental state in which the organism finds itself; whether placed under conditions of oxygen starvation though still provided with nutrients, or having to contend with life among old crowded mycelial strands, with decreased nutriment, reduced oxygen supply, and the accumulated metabolic products. Under some such condition in the tissue not yet exactly definable, the mycelial segments appear to be extremely abbreviated, though apparently growing briskly. The individual mycelial cells may thus be found detached or still connected as budded individuals. The conidial heads are usually seen to be pigmented and encrusted with chemical salts. Similarly, perithecia also contain considerable amounts of pigment and salts which are particularly readily picked up under polarised light, or when subjected to the Feulgen reaction. In the more chronic lesions most of the organisms appear merely as shells, or large doubly contoured organisms, scattered in the visceral lesions in the liver and (Plate 36, Fig. II) the kidney. They resemble the "shells" of cryptococcus in epizootic lymphangitis of horses. A mononuclear reaction is one of the common features of these lesions.



Fig.I. Section of a blood vessel in the kidney. Natural case. Stained with Claudius, showing aspergillar heads growing in the crypts or folds of the endothelium and projecting into the lumen. In individual heads two zones can be recognised, the lighter and narrower peripheral and the darker central zone, and sometimes the stalk can also be traced to its origin in the vessel wall. X 260.

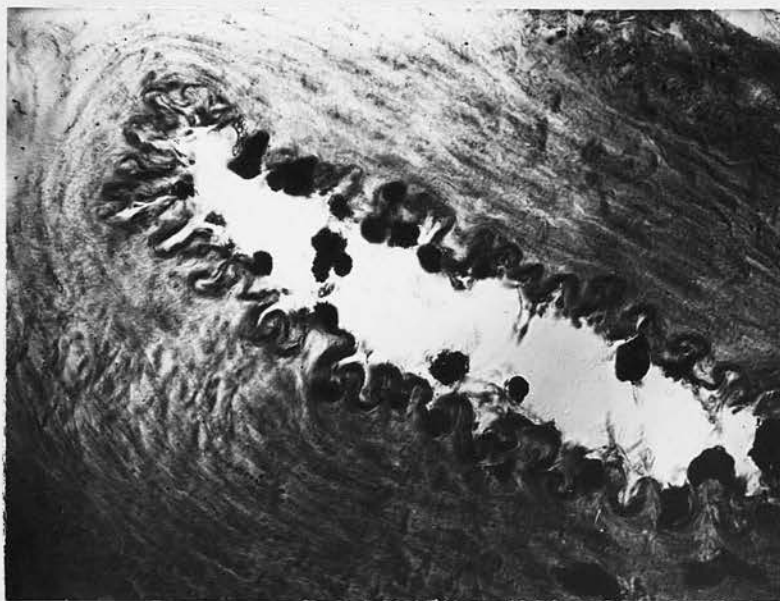


Fig.II. Similar to above. The suggestion of the mycelial strands passing across the contracted lumen can be seen.

In stages of the disease the fungus mycelium continues to extend its invasion of the bladder tissues but seems to lose the power of forming reproductive bodies and of thus multiplying rapidly. In order that the organism may continue to multiply and occupy increasingly more and more of the invaded tissues, the favourable conditions for vegetative growth are apparently present in the tissues. Sometimes in chronic cases a relationship appears to exist between the mycelium and the connective tissue, the nature of which is so intimate that it is almost suggestive of symbiosis. Considerable mycelial activity may go on in the deeper parts of the bladder wall, particularly in the muscular layer, and between the muscular layer and the serous coat, and yet no blood is passed in the urine as the mycelia have not extended in the direction of the submucosa and the mucosa. The fungus, however, produces extensive damage. The risk of the bursting of the bladder wall or of internal haemorrhage is greatly aggravated, though in the living animal the absence of haematuria gives the wrong impression that the animal is perfectly healthy. This finding explains the sudden deaths from the bursting of the bladder wall in the apparently "hale and hearty" individuals recorded in literature.



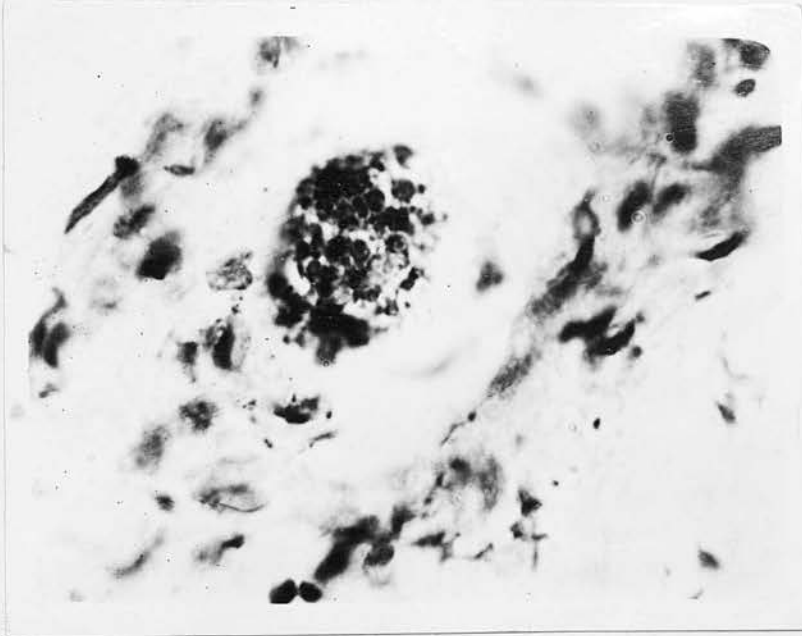


Fig.I. Section of the kidney from a natural case. Shows in the field a large body containing numerous smaller nucleated bodies. It is not clear what these bodies represent. X 1175.

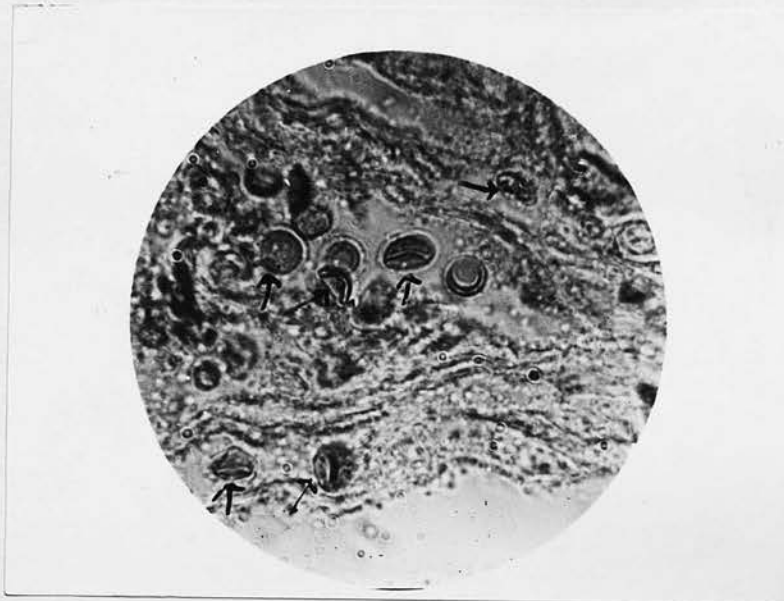


Fig.II. Section from the kidney and bladder from haematuria cases, stained with Ziehl-Neelsen or Claudius, show up in some cases cryptococcus-like rings or half-moon bodies. These have been seen by other haematuria investigators.

### Other Organs.

Degrees of macroscopic lesions are invariably present in the kidneys and the liver, and sometimes in other situations such as the lungs, lymphatic glands, the intestines, air sinuses of the skull etc.. Some evidence of the presence of the disease and of its causative fungus is manifest practically throughout the body, represented by areas of haemorrhagic extravasation, focal or diffused cellular infiltration and varying extents of granulomatous changes.

In regard to the parasitic agent as seen in the different organs, it may be added that the description already given under the bladder applies in a general way to that in all the tissues. It is to be noted that while different forms of aspergillosis have no doubt been studied by a number of workers there is no indication as to whether the different stages of the aspergillus known in artificial cultures, occur in pathological tissue. Nor is there any indication as to the relative frequency with which the different stages actually occur in each organ. It is extraordinary that literature provides no clue as to whether conidiophores and conidial production can take place in animal tissues. Even in the case of the commonest and the most serious of all forms of aspergill-

osis, namely that affecting the lung of poultry, there exists only a solitary but unauthenticated statement that conidiophores may be produced in such areas of the lung as may be exposed to the air. Regarding the nucleus of aspergillar cells it may be stated that it is comparatively large in comparison to the cell. An interesting statement, which as far as the writer can recollect, was made by Guilliermond, to the effect that the nucleus of yeast is not distinguishable from the nucleus of other organisms, notably from those present in other fungi.

#### Kidney.

The presence of lesions in this organ are practically constant. A peculiar type of cloudy swelling is often found, even in tissues collected warm and fresh. Whether this is indicative of a toxic action has not been clearly proved. Areas of haemorrhage and abscess formation appear to be more marked in the cortical and medullary regions. The kidney capsule shows localised thickening and areas of depression. Considerable parasitic activity may be present in the cortex, and particularly under the capsule of the organ. The subcapsular abscesses may become more or less organised. Malpighian bodies are enlarged and contain exudative material. The glomeruli are markedly altered, and may be largely obliterated in some places, with

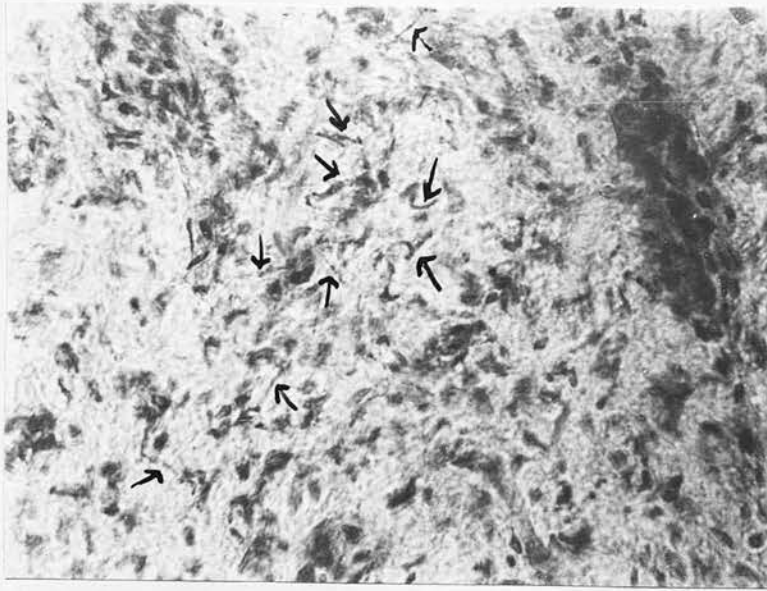


Fig.I. Section of the kidney. Natural case. Stained H.E. showing germ tubes of the fungus in an extremely fibrosed kidney. X 530

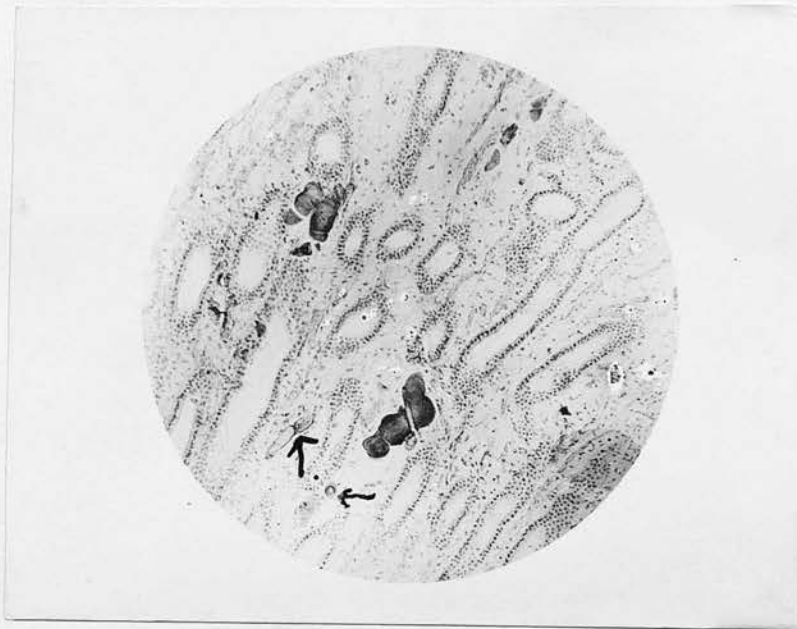


Fig.II. Section of the kidney. Natural case. Stained H.E. showing calcareous deposits lying in between tubules. The nucleus of these deposits has sometimes been found to be portions of segmented hyphal tubes. The bottom lefthand side of the field shows a part of such a segmented tube.



the Bowman's capsule lying empty. The glomerular tufts may be degenerated, partly hyalinised, and undergo degrees of fibrosis. The epithelial cells of the tubules show marked autolytic changes, and may be actively parasitised. Degrees of desquamation and shedding of the epithelial lining take place, and in places the markedly dilated tubules may be altogether devoid of any epithelium. The tubules may be impacted by the fungi, undergo marked fibrosis, and these changes are more pronounced in some parts than in others. In well-established cases, marked interstitial nephritis is also sometimes found, together with the glomerular and tubular changes. Generally round cell infiltrations are found localised in foci, but they may be uniformly distributed in some regions. In acute cases, haemorrhages occur into many of the tubules, affecting chiefly the convoluted tubules and the descending loop of Henle. Calculi may be present in numbers and aggregations in between the tubules, and also in the pelvis. The pelvis shows thickening of the mucosa, and considerable epithelial proliferation. The degree of distension of the pelvis varies to a large extent and may assume extreme limits due to hydronephrosis, haemorrhage or both. Blood vessels show marked thickening of the wall, and engorgement. Stained with methods such as chromic acid, haematox-

ylin and light green, or with Feulgen, haematoxylin and light green, the kidney tissue reveals the remarkably widespread distribution of the fungal colonies in it. Aggregations of the spores and colonies in the subcapsular region, in the parenchyma, inside the tubules, glomeruli, and in the blood vessels are thus clearly brought out. The most striking picture is however presented by the blood vessels which show the presence of numbers of characteristic Aspergillar heads. The stalk can be traced to the endothelium, and the vegetative mycelia into the vessel wall or in the perivascular region. The head is found to be composed of two regions, the outer being the region of sterigmata and the central and larger being that of the vesicle. (Plate 35, Fig. I). It is generally in the larger sized vessels that the heads are found in numbers but occasionally they may be present in the smaller-sized vessels as well. These conidial heads are readily shown under polarised light. (Plate 40). It is not easy to interpret the stage represented by all the various fungal structures encountered in the organ. Some of shrunken or empty shells seen are no doubt the conidia. (Plate 36, Fig. II). Large and double contoured bodies, which enclose numerous small and what appear to be nucleated structures are also seen. (Plate 36, Fig. I). They may or may not be the develop-

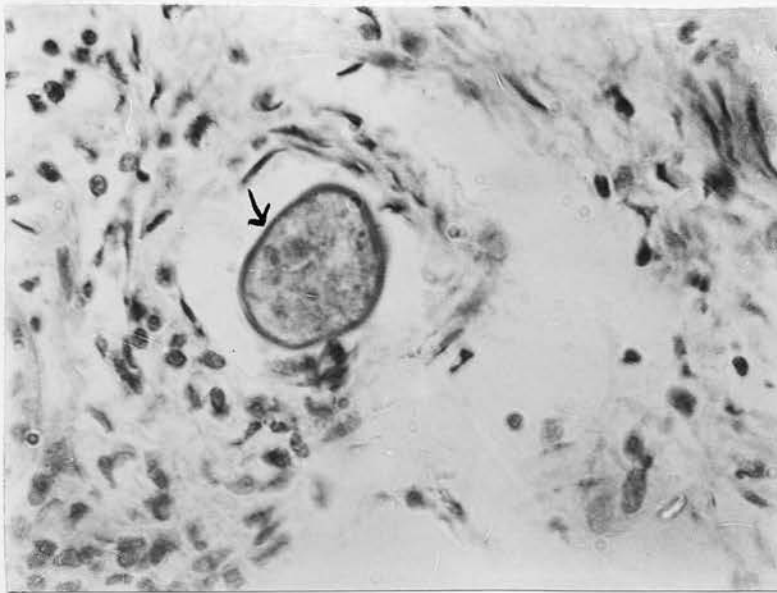
Plate no. 38

Fig.I. Section of kidney from haematuria case. Stained H.E. shows a large double contoured parasitic 'cyst'. It is difficult to state which particular stage is represented by it. Measures 30.9microns.X 680.

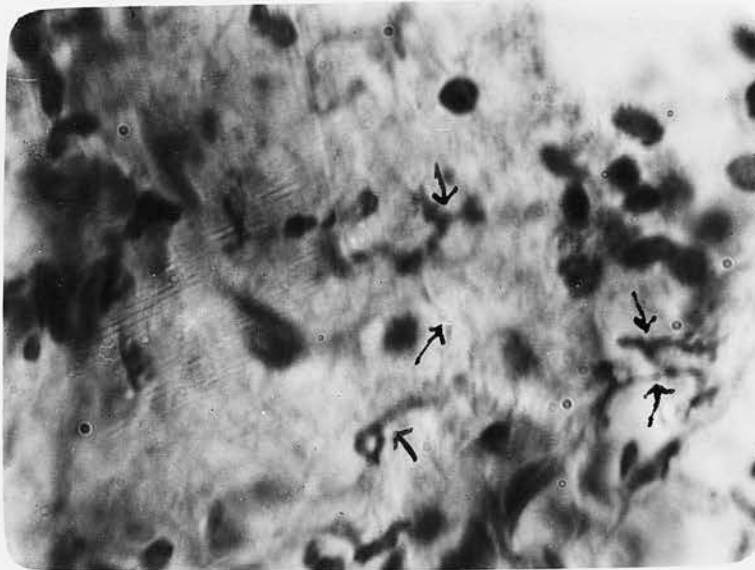
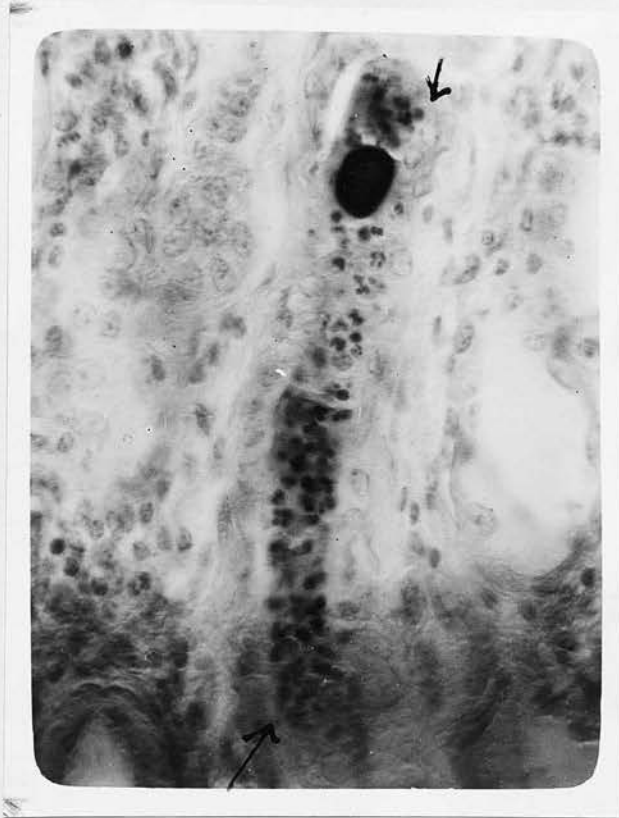


Fig.II. Section of kidney from natural case. The organ was highly fibrosed. Field shows commencing activity of germ tubes of the fungus. X 1175.

ing perithecia, or some kind of a transitional vesicle. Hyphal tubes in stages of formation, (Plate 37, Fig. I & Plate 38, Fig. II) or partially disintegrated segments which may be encrusted with salts are often found. Calculi have also been found by most workers, but no indication of what goes to form their nucleus has been offered. In the present studies, hyphal tubes have often been found to form the central nucleus of inter-tubular calculi. (Plate 37, Fig. II). Another peculiar finding is the presence of what appear to be broad hyphal tubes, packed with numerous rapidly multiplying bodies inside it. Somewhat similar pictures have been seen in cultures of the organism. (Plates 39, 57, Fig. I. & Plate 58). Mucoid cysts are found on the surface of the organ, and also in the parenchyma. Some and perhaps all of these are formed by the distension of the tubules, and a pure culture of the fungus has been found to be present in these cysts. Regarding the cellular infiltration, mononuclear leucocytes may be scattered in the organ but the polymorphs which are present in somewhat smaller numbers appear to be generally restricted to certain centres of activity. The fungal elements are invariably present in the areas of focal cellular infiltration.

In other parts of the urinary tract also, similar minute haemorrhagic lesions, focal cellular infiltrat-



Plate No. 39

Section of the kidney from a natural case. Showing imbedded in the tubule a column of fungus, probably a hyphal tube. The interior of the tube shows at the top a large sized ovoid body, while in other parts it is filled with numerous smaller bodies. It is not possible to ascribe the real biological significance to these bodies at present. X 320. cf. Plate 57 Fig.I.(a)

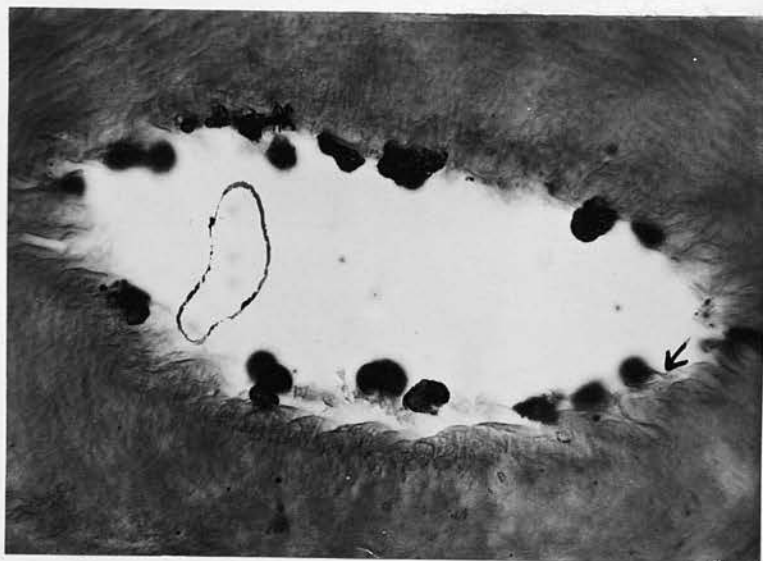
Plate No. 40

Fig.I. Section of kidney. Haematuria clinical case. Blood vessel showing Aspergillar heads with salt encrustation. Stalk of the conidiophore shown with arrow. X 250



Fig.II. Same as above, taken under polarised light. Crystals of Calcium oxalate.(?) X 250.

ion, and the engorgement of vessels in association with the fungal structures are also seen. Papillomatous growths have been found at the commencement of the ureters in the pelvis. In other parts, the ureters may show areas of congestion and minor degrees of proliferation of the lining membrane. The changes in either the male or the female genital organs which may co-exist may be of a minor degree but sometimes these are pronounced.

#### Liver.

The parenchyma appears to have undergone a degree of autolytic changes. Lesions are generally localised, and they are represented as areas of cellular infiltration, pools of blood, abscesses, and fatty changes. The haemorrhagic patches may be sometimes quite extensive. Nuclear fragmentation is marked in places. The lesions in the subcapsular regions are generally larger and more pronounced. Shrunken and crescentic spores, some provided with short sprouts are found. Aspergillar vesicles are frequently seen in the lesions. Occasionally aspergillar heads are also detected in the blood vessels, in the Glisson's capsules and sometimes in the central vein of the lobule. Fibrous wall and calcareous deposits are often found in this organ, representing degenerating hydatid cysts.

Serous Coat



Mucous Coat

1. Urinary bladder from a natural case.  
An extremely degenerated and necrosed bladder lying on its mucous surface, showing an extreme fibrosis and thickening of the blind end of the organ. The tissue was very friable.

Mucous Coat



Serous Coat

2. Same specimen as above, representing the worst case of the disease encountered in the present investigation. The bladder cavity was practically non-existent, being filled with somewhat spongy, crumbling tissue. The hope of effecting a therapeutic cure in such cases cannot ever be entertained. It is surprising that the animal was able to live in tolerable health in spite of these extensive lesions.



Lung.

The organ may be markedly congested and show areas of haemorrhage. Peribronchial and perivascular irritation are often found, and blood vessels may be generally engorged. Minute and sharply circumscribed mycotic lesions are sometimes detected, but no typical "actino" forms, as frequently found in experimental lesions, have ever been detected.

Spleen.

The examination of this organ is somewhat difficult. Multiple haemorrhages and the increased activity of enlarged and distorted follicles are common, and varying degrees of fibrosis of the organ are often seen. Stained with chromic acid, haematoxylin and light green, or Feulgen and light green, the distribution of the fungal colonies in the organ is clearly brought out, though under the ordinary staining methods no suspicion of the fungal agent may even be raised. It is remarkable that in his recent book on Splenic Aspergillosis of man, Gibson(1930) has failed to settle finally any of the outstanding controversy regarding that form of splenomegaly. It is obvious that the absence of any satisfactory method of demonstrating the organism has largely militated against his well-directed endeavour.

Intestine Serous Coat  
2Liver  
1.Kidney  
3.Kidney  
4.Intestine-Mucous Coat  
5

Tissues and organs from a natural case of haematuria. The colour has largely faded after one year's formalin fixation. The gross lesions in them can still be seen to some extent. These lesions are invariably present in all cases, but their relation to haematuria never appreciated.

1. Liver surface shows localised haemorrhagic lesions of various sizes. Around the large central lesion, a patch of minute lesions is to be seen.

2. Serous coat of intestine shows chronic nodular lesions.

3. & 4. Kidney surface shows depressions, collections of minute lesions, and individual pimple like lesions.

5. Mucous surface of the intestine shows a few small haemorrhagic ulcers.

Microscopic examination of the lesions in each case revealed their mycotic nature.

### Small intestine and Caecum.

The fungus has been detected in both the mucous and submucous coats, and sometimes even in the deeper structures. The parasite appears to stain more readily in the intestine than in the bladder. Occasional polypoid growths have been found in the duodenum, and extremely rarely in the caecum and in the terminal portions of the large intestine. The fungus has been found vegetating and even sporulating in between the intestinal villi. In some cases the mycelial activity in the mucosa has been very considerable. Only minute ulcers, with or without haemorrhagic deposits have been detected. (Plate 42). In the submucosa colonies with attached chlamydospores, resembling those of the bladder, have been frequently seen. In some cases degrees of fibrosis and cellular infiltration in the muscular coat may be present. Deeper down in the serous coat, highly fibrosed localised nodular lesions may occur. Sometimes a degree of catarrhal enteritis is present over large areas. In these studies, it has been found that though no obvious gross lesions may be discernible, histological examination frequently reveals considerable degrees of mycotic activity and the production of spores in the mucous and submucous coats.

### Mesenteric and other Glands.

Haemorrhagic lesions and enlarged follicles as-

sociated with fungal activity have been detected in some cases. In extensive cases, similar lesions are found in other glands particularly the bronchial, and the mediastinal glands.

#### Mucoid Cysts in the Air Sinuses.

The ciliated epithelium above its surface shows at different places a number of minute conidiophores. Immediately below the epithelium and occasionally in the epithelium itself, large double contoured bodies enclosing smaller structures are found. In the thickness of the covering membrane of the mucoid cyst, the blood vessels may be engorged and considerably enlarged. Besides the membrane is markedly thickened in places. It shows the presence of what appear to be transitional vesicles, being rounded bodies which radiate out all over their surface numerous tube-like structures(sterigmata)providing a clue to their real nature. The occurrence of mucoid cysts in the air sinuses of cattle do not appear to have been reported before, in any case never in the haematuria literature. The successful reproduction of young lesions in this situation, effected with the intravenous administration of the *Aspergillus* culture (Plate 43 Fig. 9 ) is certainly of significance.



Mammary Gland.

The mammary gland has been examined so far from only one case. The animal could not be of any use to the owner, as the organ was altogether diseased and profoundly altered. Histologically, the glandular structure was highly proliferated. There were extensive areas of degenerated and necrotic tissue. Masses of hard coagulated milk were interspersed here and there between the tissue. The blood vessels were greatly dilated and haemorrhagic. The epithelial cells were not only highly proliferated, but appeared to show an infiltrating character of spread. Curiously metastasis into the neighbouring organs had not taken place. The fibrosis of the organ in some parts was very great.

## VI. HAEMATURIA TRANSMISSION EXPERIMENTS.

### (i) Position in Literature.

In summarising the work of previous investigators in this connection, it may be said that negative results have been the rule, and cases where successful transmission were claimed are very few indeed. Many workers have made these attempts at transmission. In these tests a variety of suspicious materials including diseased tissues, urine, blood, samples of water and vegetation have been employed. Besides some commercial acids have also been tested. The routes of administration have been varied but positive results have not been forthcoming.

In the British Isles, Craig and Kehoe (1923 and 1925) carried out a series of experiments with both blood and urine, and they were led to conclude that no microbe or parasite was involved in the causation of the disease. In East Africa, Kearney (1918) inoculated large quantities of blood into healthy animals of different ages and breeds. Samples of Haematuria urine were injected into the bladder of a healthy cow. Scrapings from the bladder growth collected immediately after death were inoculated into guinea pigs. Negative results were reported in each case, besides no incriminating organisms were detect-

ed on bacteriological examination of the tissues from diseased subjects. The above instances of experimental tests may be taken as representative of all the work carried out so far.

When Detroye (1891, 1904) claimed the first positive results, Moussu came forward to test out the effects of the culture of the alleged pathogen which the former had supplied to Nocard. Careful scrutiny was made, and the tests carried out by Moussu failed to confirm the conclusion of Detroye that the disease was microbic, inoculable and contagious. Further the criticism was also made that Detroye had not differentiated between haemoglobinuria and haematuria. It is however interesting to note that Detroye cultivated his microbe on such liquid media as urine, serum and bouillon, and that the organism, said to be a micrococcus, thus assumed the form of a diplococcus or a streptococcus. Very young cultures of this organism appear to have been used by him in the transmission experiments, presumably on the view that the microbe happened to be a bacterium. Twelve animals were inoculated, subcutaneously and intravenously, and positive results were reported in six of them in between 3-20 days.

In face of the fact that Detroye's results could not be confirmed, it may not be admitted that

he had actually encountered a pathogenic organism. In the instance of bovine haematuria, however, technical reasons exist why<sup>a</sup> similar divergence in the results of transmission experiments may have been inherent in the problem itself rather than a matter for the absolute discredit of his work. Detroye did not record the detailed and specific characteristics of the micrococcus dealt with, and the mere morphological resemblance to a diplococcus or a streptococcus that he mentions is capable of another interpretation. (vide Plate 58, Fig. II).

A series of transmission experiments, which received an even greater prominence than Detroye's work, was carried out by Hadwen. Like others, he also had first failed to transmit the disease to healthy animals, by cohabitation with clinical cases, by injecting haematuria urine into the bladder, by giving it by the mouth, and by the injection of the blood and urine under the skin. He employed as many as seventeen animals for the purpose. While examining urine samples from clinical haematuria cases, he encountered crystals of calcium oxalate and presumed that these must have come from oxalic-acid-bearing plants ingested by the animals. Therefore he carried out a second series of experiments (with commercial oxalic acid and calcium oxalate), and claimed to have produced the disease by (i) injection of calcium oxa-



late crystals in aqueous emulsion into the bladder and (ii) by the oral administration of oxalic acid, in about five months, and two and a half years respectively. Hadwen's oxalic acid theory had two special merits, firstly that it had the possibility of offering an explanation of the connection of the disease with special enzootic areas, and secondly some workers were able to find calcium oxalate crystals in haematuria urine. Hadwen's conclusions were generally accepted and held the ground until comparatively recently. The Veterinary Bulletin, a publication of the Imperial Bureau of Animal Health, appeared to give credence to it as late as 1931.

Among the other lines of work, the possibility of plants, endemic to the haematuria areas, producing the disease due to their toxic properties has been subjected to experimental tests by several workers with a negative result in each case. For instance, Cleland (1911) experimented with Homalanthus populifolius, Indigofera australis and Goodia lotifolia, and Hadwen employed in his tests extracts of Dicentra, deergrass, the extract of alder and bracken.

With a view to explaining the negative results obtained, Hadwen remarks that it is very improbable that one special plant causes the disease because the vegetations in the various countries differ so much, and that a group of plants may thus be responsible

rather than one particular species. In Germany the general belief existed for a long time among owners of "red farms" (haematuria) that the disease is somehow transmitted from animal to animal. To test this belief, the other suggested causes and particularly the bacterial theory, Schlegel carried out experiments designed to provide clear results. 80-200 ccs. of blood collected from the jugular vein of clinical cases were administered either whole or defibrinated by one or more routes into healthy animals, haematuria urine, 50-180cc., being given at the same time either orally or subcutaneously. These administrations were repeated several times, and the animals were kept under observation (pulse, respiration, temperature and the character of urine) up to eleven weeks. Further some of the animals were drenched with three-quarters litre of urine. Schlegel reports that in these animals no abnormality was noted in life or at autopsy in spite of the large doses being repeated five or more times. Similarly 65c.c. of a filtered emulsion of verrucose bladder growths and of kidney from a clinical<sup>case</sup><sub>A</sub> was given subcutaneously into a cow, and five weeks afterwards when the experimental animal was sacrificed, no changes were discovered at the seat of the inoculation or elsewhere. In one of the two experimental healthy cows, the calcium content was found to decrease and the phosphoric acid

content to increase.

It is a remarkable fact that in the autopsy of his experimental subjects, "lesions of tuberculosis" are said to have been found in most cases. It is curious that Schlegel should not have offered any explanation for this unusual finding in his animals. It appears more than certain that he relied wholly on the gross characters of the lesions to arrive at that diagnosis, and that the lesions were actually pseudo-tubercles.

In the small animal tests, four white mice, three guinea pigs and rabbits were inoculated with one, two and five cc. of strongly bloody urine at the root of the tail, but no reaction was noticed in life or on destruction. From the above experiments and the failure to find any parasites in the postmortem examination of clinically affected cases, Schlegel concludes that the disease is not transmissible, is not contagious or infectious, and no parasite is involved in the causation. Further to test whether any general poisoning through acids produces haematuria, commercial acids and spring water from notorious localities were tested experimentally. Schlegel administered to healthy cattle increasing doses and varying concentrations of nitric acid (up to 65cc. of 0.17 to 0.39 per cent solutions), silicic acid (solution containing 50 grams of potassium

silicate in 0.29 to 1.06 per cent solution). The acids were given in water and over a length of time.

No changes were noticed in these animals.

Schlegel therefore concludes that these common acids do not cause haematuria in animals, and that the water from enzootic areas do not contain any virus or bacteria capable of transmitting the disease.

In the interesting cattle feeding experiments on the red water farm at Milner B.C. twenty cattle were placed on the farm, and grouped according to the feed or water supplied to them, with the object of determining the effect of such on the production of the disease. Nothing of significance occurred for twenty-six months, but later on nine showed symptoms of haematuria. It is to be noted that in eight of the nine clinical cases, the symptoms appeared within a period of from twenty-eight months and forty-one months, and in the ninth case forty-eight months after being placed on the farm. Three of these cattle were slaughtered and apart from typical lesions in the urinary bladder, no other constant pathological condition was noticed. This fact was substantiated by numerous autopsies in the field. Of the remaining cattle which did not exhibit blood, six were slaughtered after being on the farm for thirty-six months.

The bladders of three showed minute haemorrhagic spots, suggestive of lesions in the very early stage,





Internal organs of an experimental healthy bull, which died in about five weeks after the intravenous administration of a pure culture of Aspergillus flavus series, recovered from a Cow "Gaiety" at Kalimpong. Specimens photographed after formalin fixation of over a year. The natural colour has thus been 'bleached'.

1, 2 & 3. Portions of kidney - showing the greyish aggregated elevations and surface irregularities. Haemorrhagic pimples, and the hollowing out of the organ may be seen. Lesions in natural cases are similar.

4 & 5. Pieces of the liver. The organ was highly abnormal in colour ('washed-out' and fatty) consistency and the appearance of the whole parenchyma. Haemorrhagic and greyish lesions were widely interspersed in the organ.

6. Piece of the spleen. The organ was somewhat enlarged. Shows the haemorrhagic and greyish lesions. Mild but similar lesions may be detected in natural cases but it requires very close examination.

(7) Portion of the epididymis. The lesions resembled cold abscesses, varied in size, were very prominent to the naked eye. The localisation in the epididymis reminded one of similar lesions in human tuberculosis.

8. Intestinal mucosa showing minute haemorrhagic ulceration (cf. Plate 42, Fig. No. 5).

9. Mucoid cyst in the maxillary sinus. (cf. Lesions in natural case Plate 4 ).

10. Lung surface. Numerous minute pseudotubercles showing through the covering membrane.

11. Lung parenchyma riddled with lesions of varying sizes. In natural cases of haematuria comparatively few localised nodules are only found.

For the urinary bladder and appearance of liver parenchyma from this case see Plate 44 below.

and another showed slight but quite definite lesions. The cattle which showed positive or suspicious evidence of disease had all had access to pastures on the affected farm. These pasture fields had a good deal of surface water standing on them at certain periods. Of the nine positive cases, one received hay and grain from non-red water farm, and artesian well water, but was allowed pasture grazing. The other eight received hay from red water farms and grain from non-red water farms; of these again four received artesian well water, and had access to surface water in the pasture fields and to stream water. The evidence gathered from these interesting field experiments indicated that the disease is contracted on the pasture fields on haematuria farms.

In order that more specific data might be secured regarding the environment or of the factors responsible for the disease, certain alterations were made in the grouping of the experimental cattle. Ten more cattle were added to the Milner herd, and two animals were put into each of the five groups, receiving different combinations of feed or water supplied from the red water farm, and feed from non-red water farm, either kept restricted to the stable or allowed on the pasture. All straw for bedding came from non-haematuria farms, but up to the date of reporting no haematuria was seen. Besides, two yearling heifers

were kept on a neighbouring farm in a yard which was suspect because of the considerable loss from the disease which had been experienced among the bulls that had been kept there. It was reported that some of these bulls contracted haematuria without ever having been on pasture. The yard had no vegetation on it but as regards the presence of surface water for long periods due to heavy rains it presented conditions closely resembling those of the haematuria pastures. Feed from non-haematuria farms, artesian well water, and surface water from the yard were available to the heifers, but up to the date of report they had not produced any evidence of the disease. In another series of experiments, guinea pigs were fed urine from clinical cases and a pronounced cystitis was thus produced, and these lesions resembled very much those of haematuria. The controls as well presented some lesions. The results could not therefore be interpreted. Further two calves were fed with haematuria urine for about a year and had consumed twenty gallons of urine between them during the period. The experiment was in progress till the time of writing.

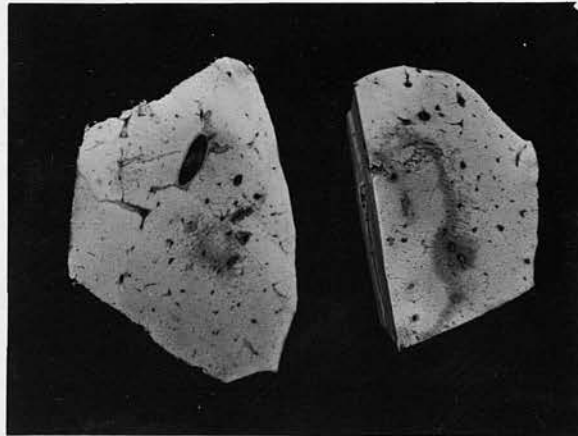
It has been noted above that Detroye, Hadwen and Bankier are the only workers who have reported successful transmissions. Of these, Bankier's report appears to be the only one pointing to some clear clues and suggestions. Most of the investigations,



Internal surface of the  
Urinary Bladder



Liver Parenchyma



Urinary bladder from an experimental healthy bull, subjected to a massive intravenous dose of pure culture of Aspergillus flavus series (recovered from the bladder tissue of an Ayrshire Cow). Note the minute but well-established haemorrhagic lesions, also nodular growths. The urine at autopsy was positive for blood chemically. For other organs see Plate No. 43.

carried out so far in different countries, have been concerned mainly, if not exclusively, with field observations. While these field observations may establish a definite relation of the pasture and forage, etc., it will be realised that the final elucidation must depend upon some positive work with morbid materials from clinical cases.

## (ii) PRESENT STUDIES

### Material and Methods.

In planning future experimental transmission work, past experience demands that a greater appreciation of what constitutes a positive result, and what results are to be expected from the methods adopted, and how those methods are to be utilised to the fullest advantage are points of fundamental importance. No one doubts that under natural conditions the disease develops very slowly, and the latest data regarding early lesions and incubation period produced in the field observations at Milner B.C. should be kept in view. Past workers have repeatedly made the mistake of expecting rapid results on the preconceived notions of a bacterial infection, and there are reasons to believe that such lesions as were present were summarily ignored during the making of naked-eye search for gross lesions. Every natural disease has



Fig.I. Section of the bladder. From a bull in which the disease was successfully transmitted, to show the manner in which the muscular tissue is altogether 'eaten away' in parts, with considerable thinning of other parts. Massive amounts of mycelial felts replace the muscle strands, but they have not been clearly brought out in this preparation. X230.

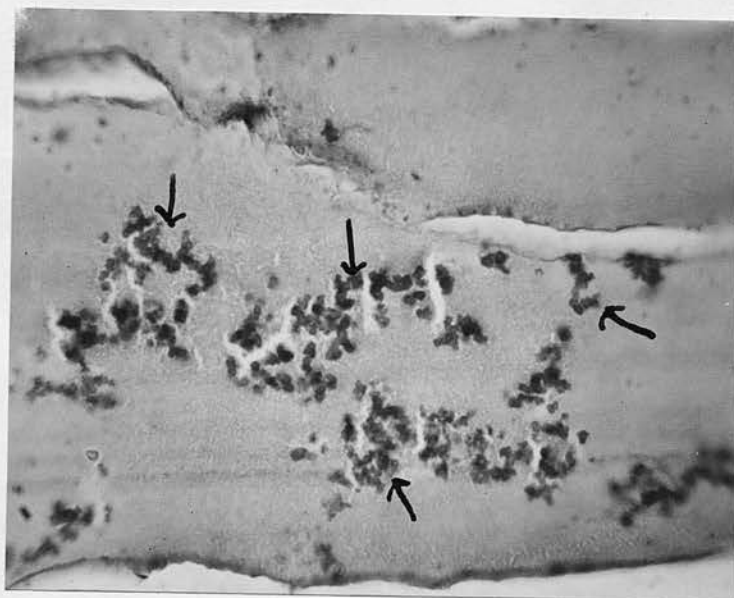


Fig.II. Section of muscular tissue from the bladder of a bull. H.E., Case of successful transmission, showing the peculiar character of the fungal growth in the depth of the muscular tissue, preponderating in certain types of nutriment but containing only traces of others, like the somewhat semi-anaerobic conditions. In their actively growing state in highly haemorrhagic areas, these 'knotty' organisms may only take the eosin stain and resemble fibrin. A drop of caustic potash readily shows their nature. X650.

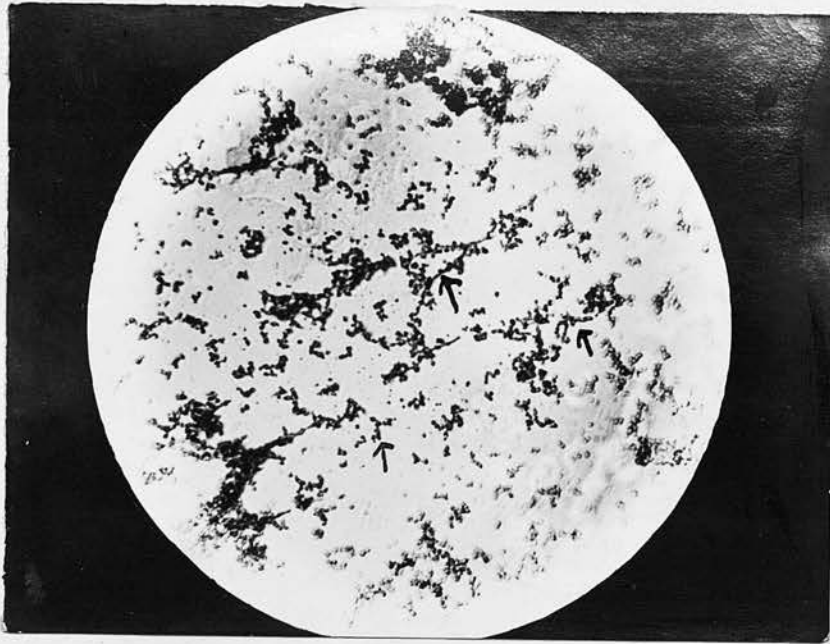
Plate No. 46

Fig.I. Earlier in the investigation, smears made from heart blood of white mice, succumbing to subcutaneous inoculation of haematuria urine sediments after a period of about five weeks in each case, showed the above picture. The strings of chained material were then ignored as fibrin filaments, but after potato-kidney cultures were studied in sections they were found to be a transition stage of the fungus.



Fig.II. Same as above. From another mouse. Compare the organisms with those in sections of potato in Plate No.57, Fig. 2 ..



Plate No.47

Specimen of urinary bladder, turned inside out, photographed immediately on collection, from a case of successful transmission in a bull. The animal developed haematuria a few days before destruction. The bladder was found to have nodular lesions and a considerable amount of oedema in the bladder wall. Sections were cut and the fungus was found to be intimately associated with the experimental disease. Section of the muscular tissue from this case is illustrated in Plate No.45 ..

its earliest and smallest beginnings, which may or may not be detectable even under the microscope. Besides the features of an artificially produced disease, under exaggerated conditions of experimentation are not likely to produce the same identical composite picture, as one would find in the tissues representing a mildly progressive disease of long duration. The existence of the pre-clinical stage of the disease has come to be realised only very recently, and the absence of that knowledge could not have been helpful in the interpretation of transmission experiments carried out in the past.

The infective clinical material, used in the transmission experiments by other workers include blood from the jugular veins, the whole (haemorrhagic) urine, portions of the bladder growths or emulsions made from it. The materials were administered through all the routes, viz. subcutaneously, intravenously, orally and even injected into the bladder of healthy animals. The administrations were given either on a few occasions or over a length of time. It is remarkable that there happened to be no method by which the amount of the material to be administered, or the frequency of the administration required, could be judged or controlled. Besides what is more important is that the character of the lesions to be expected in any successful trans-

mission under the conditions of experimentation was not realised. Even the necessity of destroying animals for a careful examination of the bladder, rather than be content to pronounce the result only from the character of the urine, was not appreciated.

In some of the earlier transmission experiments, the present writer made similar mistakes and therefore his results also were not sufficiently controlled to be clear and precise. For instance, experimental animals were not finally disposed of by destruction but were utilised for other work. Sediments of urine were used in the present investigations rather than the whole urine, used by previous workers, thus obviating the administration of large quantities of unnecessary material and enabling the infective doses to be given in a greater concentration and with a frequency somewhat similar to what would happen naturally in an enzootic area. In short, no method of concentrating the infective material was adopted by the previous workers. Further, in the writer's work when the inoculum was introduced subcutaneously the nodular lesions were not left to themselves to disappear spontaneously with time, but were either surgically removed and examined culturally and histologically, or smears were prepared from the inside of the lesions, by aspirating the contents with sterile needles or from the burst lesions. Similarly, urinary sediments

from haematuria cases were injected directly into the testicle or liver of rabbits, and the organs were subjected to complete examination by collecting them.

While the testicles could be removed without sacrificing the animals, the liver was collected either at death or by destroying the animals. Another method of preparing the inoculum was by collecting the urinary sediments, washing them with changes of sterile normal saline by means of the centrifuge, and incubating the washed sediments in sterile bouillon at 37°C.

One of the other earlier mistakes in the present investigation was the exclusive reliance placed on naked-eye examinations, and the failure to take into account such lesions as were present in the bladder of destroyed animals. The writers experience shows that the microscopic examination of sections is the only correct and dependable diagnostic method, though the changes in the character and appearance of the urine as passed or after settling is a useful adjunct.

Moreover it is felt that in order to ascertain if a transmission experiment has succeeded the following important points be remembered:-

- (1) The varying features of the natural disease in the early commencing, and in chronic cases to be kept in view. (cf. Bankier's findings)

- (2) Naked eye examination of the mucosa and the deeper tissues by sectioning through the bladder wall at



several places.

- (3) Direct examination of smears made by impression and scraping.
- (4) Microscopic examination of the urinary sediment.
- (5) Materials for close histological examination should be representative.
- (6) Methods of staining must be appropriate.
- (7) Material for fresh examination should be suitably prepared.
- (8) Culture from the depth of the bladder tissue after superficial treatment with disinfectants.
- (9) Culture from jugular blood or from heart blood.

(a) Small Animals.

Among the ordinary laboratory animals, rabbits, guinea pigs and white mice were largely used by the writer. When the suspicion arose that the haematuria parasite might be an entamoeba, it became necessary to employ dogs and cats as experimental animals. A number of these were used, but they were found to be unsuitable for haematuria investigations. The dogs were given (by the mouth) urinary sediments, and were injected with blood from rabbits succumbing to similar administrations, but they continued to be apparently free from malaise. Besides the oral administration, the cats were given urinary sediments intrarectally, and the majority died after varying periods of up to eight weeks, showing in most cases degrees of localised ulceration in the intestine. A few of the cats however lived for eight or nine months after the initial feeding. Since only negative results were obtained with regard to the suspected entamoebic infection, and due to the difficulty of handling semi-wild cats which only could be made available for these tests, other species of animals were requisitioned for the subsequent experiments.

About this time wild rats became a serious pest in the laboratory area and large numbers were trapped. As these were awaiting disposal, it was thought advis-

able to carry out some feeding experiments and subcutaneous injections upon them. Circumstances however supervened, which prevented any further use being made of these extremely economical animals. Firstly the rats began to die in captivity, and secondly, those that survived were found to be already carrying in their <sup>a fungus infection</sup> system, and discharging numbers of Grampositive large, and ovoid yeast-like bodies in their faeces.

When white mice were however used the results obtained were striking. For instance, when in some of the earlier experiments a small dose or doses of urinary sediment, after a preliminary preparation, <sup>(p.263)</sup> or directly, were given into the subcutaneous tissue of white mice, it was observed that the majority of the mice so treated succumbed in about the same period of four to five weeks, revealing some mild but distinct lesions in the kidney or elsewhere. A further observation made in the mice was that when emulsions of infected internal organs were used as infective material, the period of time required to produce fatal results in mice was gradually shortened till the mice began to die in two to nine days. Smears from the heart blood from each of these cases showed very numerous branching, Gram-negative chained organisms. <sup>(Plate 46)</sup> Smears from the liver and other internal organs showed minute budding organisms, some of which were tending to take the Gram

stain. Bacteriological cultures were made from the heart blood and the viscera of these mice, but no significant bacteria could be recovered. In order to see if there existed in the internal organs of these rapidly dying mice any lethal factor for bovines, four healthy bulls were either drenched, or inoculated subcutaneously or intravenously, with emulsions of internal organs from these mice. In about a year's time it was thought that the bulls had not 'taken'. Samples of urine from these bulls were examined by the naked eye and chemically, for the presence of blood with negative results. Most of these animals were transferred to another experiment, and when they were eventually killed, two of them presented unmistakable signs of bladder disease. Histological sections from one showed lesions similar to some extent to those of the natural disease. Although at the time it was proposed to repeat the experiment with similarly prepared mouse material, more direct results became available, and this idea receded into the back-ground.

Regarding the rabbits used in the early experiments, it may be said that they were given urinary sediments into the subcutaneous tissue, or into the testicle or the liver. Injections into the liver produced abscesses in that organ which penetrated through into the diaphragm. The animals died within seven to ten days, and numerous cryptococcal forms of the parasite



were detected both in stained sections and smears. The liver tissue was highly degenerated and necrotic with areas of haemorrhage and cellular infiltration in the parasitised parts of the organ. Similar lesions were present in the testicular tissue. It was found that for the morphological study of the developmental stages of the parasite, the testicular lesions were more suitable than the liver or the subcutis. Pure cultures of the *Aspergillus* were recovered from the suppurating nodules in the testicle and the skin. In sections chlamydospores and sprouting conidia were detected. In the subcutaneous nodules, large numbers of unchanged conidia and other capsulated forms were present. The great majority were intracellular. Others were lying free. There were young and rapidly multiplying forms, empty capsules, budded organisms and others in the process of degeneration. The thickness of the capsule varied in individuals, presumably representing different developmental stages. Some spores were inclined to be more stainable than others. One of the earliest rabbits used in these tests, it may be noted, died after ten days of receiving urinary sediment subcutaneously, and was found histologically to have died of a mycotic pneumonia. In several rabbits the blood vessels of the bladder were markedly dilated, while in two or three animals a tendency to papillomatous growth was evident. In guineapigs, greyish areas in the kidney and cystitis were produced.

(b) Large Animals.

Before employing healthy bulls and calves in transmission experiments, these were first kept under observation for varying periods, usually for a fortnight or a week. To start with, the body weights were recorded. A temperature chart was maintained for each animal, showing the temperature of each morning and evening. This temperature recording was continued till the animals were finally disposed of at the conclusion of the observations. Faecal smears were examined for helminths, their ova, or for coccidial oocysts, and blood smears for protozoa. Samples of urine were examined for cellular content, presence of blood cells, and for fungal mycelia and spores. As complete an information as possible of each animal to be used, was thus secured before the formal commencement of the transmission experiments. These elaborate precautions were observed till one could be sure of the suitability of the individual subjects for the work in view.

Following the work of the previous investigators, amounts of jugular blood from clinical cases were administered by various routes to healthy animals. Portions of bladder growths, collected fresh and aseptically, were introduced surgically into subcutaneous pockets in a number of cattle. Again these growths were minced and emulsified, and introduced repeatedly

into the urinary bladder of female calves with the help of a catheter. Similar emulsions were injected into the vulval lip, or into the rectal submucosa. However in none of these animals did any marked and noteworthy results appear.

Urinary sediments do not appear to have ever been used by any previous worker, according to the recorded information. In the present studies urinary sediments from the active stage of haematuria were used. When these were inoculated subcutaneously, nodular abscesses were formed. In the majority of cases no obvious pus formation took place. The injections were given at various intervals, once or twice a week, every alternate day, or once every fortnight. It was found that considerable variations took place depending upon the frequency of the administrations, and that the abscesses were cold and not markedly painful. It was only in rare instances that the abscesses burst spontaneously, and this appeared to be due to either a too frequent or a too massive dosage. The external manifestation of the inoculation consisted of a nodular elevation or patchy thickening, but no disturbance in the body temperature or in any other normal functions was exhibited. On excising these cutaneous nodules sections were prepared and examined when considerable histological reactions were seen. It was

found that in areas a considerable number of fungal spores or conidia were phagocytosed by the body tissue cells, that a very active fray between the host and the parasite, represented by degrees of haemorrhage, degeneration, cellular reaction particularly of polymorphonuclear leucocytes, was in operation. In cases where the injections were recent or repeated, the lesions were found to have extended to considerable depths. The degree of haemorrhage in the tissue was found to be comparatively severe, and greater in degree than originally anticipated from the previous experiences of nodules set up with suspicious materials from other diseases. Regarding the parasites, occasional hyphal tubes or even shreds of mycelia apparently in the process of disintegration were observed. The fungal spores were most numerous, and appeared to be possessed of only very restricted powers of budding, since the germ tubes produced by them were in most cases highly abbreviated. The tissue elements appeared to be capable of disposing of the fungal spores, presumably only as long as the dose or numbers of the parasite were not concentrated beyond a certain limit. Stages of degeneration of the spores were also seen. About twelve animals in all were subjected to these subcutaneous injections from time to time. Cultural examination of <sup>the</sup> material, aspirated out from the centre



of the subcutaneous nodules (with the help of sterile needles), and of the excised subcutaneous tissue was made, and the Aspergillus sp. recovered. In smears made from the abscesses, intact or bursting spontaneously, or from those which were surgically incised, the characteristic fungal elements were invariably found. No actual haematuria was however produced in the majority of the animals so treated, barring the exceptions mentioned below. The information regarding the appearance and behaviour of the parasite in histological lesions which resulted from these experimental studies were of considerable value in interpreting the findings in natural lesions.

Of the animals of this lot, five revealed degrees of bladder disease recognisable by the naked eye. Hill Bull No.1061. showed patches of chronic congestion and old haemorrhage in the bladder wall. Hill Bull No.924. presented a few rounded nodular growths on the mucous membrane. Hill Bull No.143. showed signs of haematuria in life a few days before destruction. Extensive oedema of the bladder wall, rounded nodular growths and a few solidified calculi like deposits were found at autopsy. (Plate 47) Hill Bull No.193. showed distinct chronic congestion of the bladder, but it appeared to be more superficial than in the other animals. Hill Bull No.357. passed blood in the urine for some days in life. It is to be noted that

only two hill bulls (Nos. 143 & 357) actually exhibited haematuria. The definite lesions in the bladder in different stages of formation found in the other cases suggested that if the animals were allowed to live longer, outward signs of the disease might have been manifested in due course in them as well. This view appears to be reasonable in view of the known facts regarding the early stages of the natural disease.

The necessity of a complete histological examination of the urinary bladder from these cases was not realised at the time and therefore not made. Regarding the control animals, urinary deposits from healthy cattle, where such were available were collected and pooled together from several animals, washed and then were employed in the same manner as in the above mentioned cases.

Among the other bulls used in transmission experiments, two animals were given a single administration intravenously of 20 c.c. of a saline emulsion of bladder growths. Both the animals were kept under observation for about nine months till finally they were destroyed. In the meantime their body weights, temperature, results of blood and urine examination were recorded. Each of them gained in weight but did not reveal any marked change in the urine. The bladders were examined with the naked eye, and in the absence of any gross warty or nodular growths they were passed as 'apparently normal'. Two animals which re-

ceived urinary sediments into the rectal submucosa passed soft faeces for a few days after the injection. They also gained in weight and when finally destroyed did not show any gross lesions of the urinary organs.

#### Experiments in young calves.

Four calves between the ages of 5 to 8 months, of were taken, and their urine examined, culturally and microscopically, for about a week to 10 days. Two were inoculated subcutaneously with urinary sediments in normal saline from haematuria cases. In one the injections were given once in every five days, and the received injections every eighth day. The third animal was drenched with urinary sediments every third day, and the last animal received weekly injections into the vaginal submucosa. An increase in the cellular contents of the urine was often exhibited lasting for 4 or 5 days till it gradually fell down to normal. The urine of the animals was examined periodically. It was only in one calf (No.30), which received the infective material through the mouth that actual haematuria was exhibited after about 3 months of the commencement of drenching. The bleeding was not continuously but recurred at intervals for about four months of observation. One of these calves is discussed below.

#### (c) Experimental Lesions.

Among the large and small animals subjected to administrations of infective materials from haematuria

cases, lesions were detected in a number of animals of various species. Urinary sediments appeared to be more infective and suited for transmission work than even portions of bladder growths or kidney lesions. The transplantation of the growths or lesions into subcutaneous pockets or into the submucosa of the rectum and vagina has not succeeded. Emulsions of the lesions were administered only occasionally in each animal and did not set up any marked results. The presence of the infective agent, the fungal parasite, was demonstrated constantly under microscopic examination; and in the artificially produced subcutaneous nodules, the close association of fungal structures with those lesions characterised by some of the features of haematuria was invariable. It is remarkable that irrespective of the tissue into which the sediments were inoculated, the character of haemorrhage, degeneration and cellular reaction of the host, and the features of the parasite were the same, and compared very favourably with those of the natural disease. In the cats succumbing to intrarectal administrations, patches of intestinal ulceration formed a common feature, but nodular lesions were also present on the renal surface in some of them. Similarly mild but distinct lesions were present in the kidney and sometimes in the liver of the white mice. Among the rabbits a progressive disease was set up by local inoculations into the liv-



er and the testicle, and while in the former fatal results followed, the disease in the latter case was comparatively mild and localised. Subcutaneous injections also caused death of a number of rabbits, and the finding of mycotic pneumonia in one of them was very striking. Of all the species of experimental animals, the most noteworthy results were no doubt produced in the natural subjects of haematuria. Of these, two bulls and a calf actually developed haematuria in life, while degrees of bladder disease readily recognisable by the naked eye were present in three other bulls. That the artificial lesions were more or less identical with those of the natural disease was proved by detailed examination of the lesions in which fungal activity was well represented. (Plates 45 & 46). Of the other animals in which no clear naked eye lesions were recognised, one could not definitely eliminate minor degrees of infection. Here it must be explained that only small amounts of haematuria urine were collected, and as a number of these experiments were proceeding simultaneously the amount of urinary sediment available for each animal was rather meagre. It is now felt that the doses employed in each case must have been much lower than actually infecting animal naturally. Besides it is certain that the fungal elements as discharged in the urine by an affected subject are insufficiently aerated and consequently contain a far fewer number

of spores than those of the infective material responsible for the disease in nature. In surveying the past work, it is felt that if the urinary sediments were kept exposed to the air for at least a week before use the natural conditions could be more closely approximated and even better results expected than recorded here. The experience gained in these studies, particularly in mice suggests that a highly active and virulent blood form of the fungus is produced, hitherto unrecognized. Finally the case of a calf that developed a highly extensive haemorrhagic inflammation in the subcutaneous tissues, following upon injections given there, and died of a rapid diffusion of the parasite into the general system, must be mentioned. This case will be compared later to another that succumbed in a similar manner after subcutaneous injections of the pure *Aspergillus* culture.

VII.. CULTURAL STUDIES.(a) Materials and Methods of Previous Workers.

Samples of the blood, urine, bladder growths and the kidney tissue from haematuria cases have been submitted to cultural examination by previous workers (Schlegel, Hadwen). Attempts were directed primarily towards bacterial organisms present in the tissues, though on one or two occasions attempts to cultivate protozoan organisms have also been made without any success. Various media under varying conditions have been employed. The results recorded have been mainly of a negative order with a few exceptions where streptococci, coliform organisms, and other saprophytic bacteria were cultivated.

In some instances numerous cocci were seen in urinary smears, but when attempts at cultivation were made, the organism could not be grown. Detroye claimed to have cultivated a pathogenic micrococcus or diplococcus, but his results could neither be confirmed nor his organism correctly identified. A group of workers have one after another described capsulated bodies in the bladder tissue resembling coccidia, but they have not produced any culture from the cases dealt with by them. Recently Durin

and Unglas treated haematuria cases on the supposition that it was a form of Colibacillosis, but from the records of their work seen by the present writer, it is not clear if any coliform organism was actually cultivated by them. A few other workers, like Hadwen, Craig and Kehoe have carried out cultural studies, and are agreed that in the early stages of the disease the affected bladder and kidney are sterile, and have explained that it is in old-standing cases that the lesions become contaminated with bacteria. Case, however, (1911) reported the finding of a coccus-like body in some few of the red cells. While the entire number of parasites was intracellular, the extracellular ones were not numerous. With all the stains used, the parasite took a bright blue colour and its size varied from 0.5 to 3.5 microns. They were always circular, rarely in the centre and usually situated near the margin, but the organisms were not cultivated. It is possible that these were the protozoan parasite, Anaplasma marginale, though the bright blue staining with all the stains appears to be somewhat anomalous.



(a) Present Studies.

In the earliest cultural studies, attempts were directed to the cultivation of the bacterial flora of fresh samples of diseased bladders, as they became available. Inoculum was obtained from the lesions by scraping, or with the help of a swab or platinum loop. The cultural material was secured with sterile precautions from the surface, and from the interior of lesions situated at different depths of the organ. Portions of the actual diseased tissue were also employed as the "seed" material, either in small bits, minced or ground up. In some cases, a series of cultures was attempted with the material, after a preliminary steeping into 2% formalin, or absolute alcohol for varying brief periods of a few seconds to minutes, with the idea of disinfecting the surface of the material of any chance contamination that might have taken place. In short, it may be stated that the bladder tissue yielded negative results for bacteria in quite a large proportion of cases, while in others Streptococcus faecalis, Corynebacterium, Staphylococcus albus, coliform organisms, Flavobacterium, Pseudomonas, B. subtilis and other organisms of no significance were recovered. According to routine, the bacteria were subcultivated as early as possible and when pure the original tubes were discarded. In the presence of bacteria, the generally <sup>growing</sup> slow-7<sup>8</sup>

fungus, where such might have been present, had thus no chance of growing. If after forty-eight or seventy-two hours any evidence of a fungus growth in one or more tubes became manifest, the tubes were discarded on the usual supposition that these were contaminants. A certain amount of attention was given to the *Corynebacteria* cultures. The bacteria recovered were found to be common commensals in materials from non-haematuria cases as well.

As a result of the finding of certain definite "encysted" or "capsulated" bodies, where one could see something like an outer "ectoplasm" and an inner "endoplasm" in haematuria urine and bladders (Plate No. 8 Fig.I. and Plate Nos. <sup>21,23</sup> Fig.II ), it became necessary to consider the possibility of protozoan infections. The ring-shaped nuclear pattern, size, vacuolation, capability of cytoplasmic protrusions in several planes, etc., were some of the features which added strength to the suspicions.

It was ascertained that among the protozoa, *leptospira*, *leishmania*, *trichomonas* and *entamoeba* were the only parasites described as occurring in the mammalian urine. Of these, the characters of the peculiar bodies of haematuria material could, it was found, apply only to an *entamoeba*. However, similar obscure bodies found by other workers were interpreted as

Coccidia in haematuria literature. The next cultural studies were therefore directed to protozoa in general, and to amoebae and coccidia in particular. The egg medium of Boeck and Drbohlav, N.N.N. medium, Barret & Smith's medium, 2-5% potassium dichromate, sterile hay infusions, and similar other media were tried with materials from a number of cases for about two years. Only negative results were obtained, excepting in one or two cases where accidentally, a free-living flagellate appeared. However, in one haematuria case, which died suddenly and showed extensive petechial haemorrhages throughout the viscera, including endocardium, pericardium and the intestinal tract, the curious observation was the presence of teeming numbers of actively motile large trypanosomes in the haemorrhagic urine. The sample of urine was maintained in the laboratory, and the parasites were found to be alive and wriggling for the remarkably long period of about seventy-two hours in the urine. Transmission experiments in large and small animals were made without success. Attempts at cultivation failed, but from the morphological features the parasite was identified as the usually non-pathogenic Trypanosoma theileri of cattle. Instances are on record of occasional pathogenicity and fatal results from this organism. Similarly, on one occasion some developmental stage of

Sarcosporidia was detected in direct smears from the bladder lesions.

These attempts having failed, attention was directed to other possibilities. Fungus mycelia had been seen previously in bladder sections but ignored as having no significance. Transmission experiments had been started, and the experimental cattle were sacrificed for ascertaining the actual result. One of the animals passed blood in the urine, and on autopsy showed extensive nodular growths, minute haemorrhagic ulcers, and considerable oedema of the bladder wall. (Plate 47). As the bladder on cultural examination yielded a yellowish-green fungus, portions of it were maintained in 50% sterile glycerine for future use in transmission, but later it was thought desirable to examine the lesions histologically as well. (Plate 45). The glycerine-preserved tissue was therefore sectioned. Thus the effect of glycerine in the tissue was found to be interesting, in that while the tissue had lost a certain amount of its staining capacity, the fungus organisms stained more markedly. The possibility that glycerine may form a cultural medium for the fungus presented itself, and was later confirmed.

#### Methods and Media Used.

Attention was thus drawn to the fungus flora of healthy and haematuria animals, and since 1935



intensive studies from this angle have been made, utilising such materials as urinary sediments, internal organs, the jugular blood from living animals, and the heart blood from dead animals. Only sterile glass ware and instruments were used. In autopsical examination, pipettes of heart blood or other fluids were first collected, followed by any other cultural material, such/<sup>as</sup> portions of internal organs that it was thought advisable to examine. Fresh tissues were maintained in sterile ware in the refrigerator, with or without being kept immersed in 50% sterile glycerine, for use when required in the future. With a view to further sterilisation of the tissues to be subjected to cultural examination, the outer surfaces of the materials were singed with the red hot iron spatula, or the materials dipped in absolute alcohol for two minutes, or in 1 in 1000 solution of perchloride of mercury for five minutes. A loopful of inoculum was then obtained from the central portion of the organ concerned and spread out on the culture tube or petri plate. Pieces of organs from healthy cases were similarly subjected to the above techniques.

The routine media used for mycological work were employed, together with various special media found by others to be useful in certain fungus infections not readily cultivable (as in the case of *Cryptococcus*,

Histoplasma capsulatum, Coccidioidal granuloma, Malassezia, etc.). Both solid and liquid media were used, and anaerobic cultures were also attempted. In a number of cases the preliminary isolation was carried out with advantage upon special selective media, such as a 25% sterile glycerine, 5% citrated agar medium, bread paste, or potato slants. The different cultural media used included Sabouraud's agar, glycerine-agar, dextrose-agar, tartarated dextrose-agar, potato media, tea infusions, 10-25% sugar solution, Petroff's, McKelvey's, Raulin's, Czapek's, Czapek-Dox agar, gelatine, litmus milk, 25% sugar broth, blotting paper, plaster of Paris blocks, weak carrot infusion agar, prune extract agar, agar slide culture, a 5% citrated physiological saline, a 5% citrated glucose broth, and a 5% citrated Sabouraud's agar, citrated peptone bouillon (Barottee and Velu's work, with Cryptococcus sp.).

Cultures were generally made in tubes and petri plates, and occasionally giant cultures were also studied by growing the organisms in Erlenmeyer flasks. Cultures were thus made from a large number of clinical haematuria cases as they were detected from time to time at the Mukteswar Institute. As and when the cultures were first isolated in a pure state, one set was always tubed and kept paraffined to maintain them

free from any contamination. The cultures were purified either by the decimal dilution and pour-plate method, or by the multiple stroke method, the process being repeated till among the four or five plates of one series, only one or two plates showed an odd isolated colony or two. Increasing numbers of pure cultures were thus accumulated, and it was noticed that a yellowish-green type of fungus was the most frequent and outstanding culture obtained.

Samples of urine from healthy cattle were first examined culturally and also microscopically, with a view to ascertaining the extent and character of the microflora usually found in animals of a haematuria locality. Negative results only were obtained in the majority of cases. In the rare cases where any fungus cultures resulted, the fungus concerned was found to be a different kind from that encountered in haematuria cases, being either a rhizopus, a mucor, a *Penicillium*, *A. niger*, or other ubiquitous species. The urine samples of healthy cases were remarkable for their general freedom from many bacteria or fungi. The cellular content of the healthy urine was also very meagre or absent.

Internal organs from cases of haematuria were collected in special cases by destroying animals, and as a routine in all other cases that were subjected to

autopsy. All possible precautions were taken to avoid contamination. Lesions from the urinary bladder and the kidney were chosen. They were minced into small bits or used whole for planting, after a preliminary washing in an antiseptic solution.

Initially no attempt was made to identify the different strains of fungi that were being collected together by the addition of individual strains from time to time. It was anticipated that when cultures from a sufficiently large number of animals were collected by the simultaneous use of several methods, including the recovery in pure state from the heart blood of white mice or rabbits dying of experimental inoculations, the cultures of significance would automatically be sorted out as the commonest strains. Further, when the strains from all the different infected areas in the country would become available, the comparative result was expected to lead to much simplification, if the fungus hypothesis was based on substance. Eventually unforeseen difficulties developed due to the pleomorphism of the organism, the failure to use a standard medium, atypical forms, further complicated in some instances by the co-existence of somewhat nearly related contaminants or chance invaders. It was not appreciated at the time that it was essential to use Czapek's or other standard



medium. Whichever medium was conveniently available at a particular moment, whether Sabouraud's, blood agar, potato slants, 25 per cent glycerine or 10 per cent glucose broth, was used with different isolations. In spite of the difficulties, it was realised that the predominant fungus was characterised by a typical conidiophore, luxuriant production of conidial "powder", and was unmistakably an Aspergillus with a more or less greenish pigmentation. But atypical conidiophores, the presence of white colonies and sometimes a tendency to turn brown, as also the occurrence of "budded" forms, blastomyces-like, at the bottom of liquid media appeared to be confusing. Repeated tests showed that no Penicillium or "yeast" was contaminating the cultures, and sometimes free conidia were found to be adhering together in large round masses. The possibility of the fungus being a member of the related genera was therefore kept in view.

At this time one of the trained bacteriological assistants of the Institute was making a visit to the Kalimpong haematuria infected herd. This opportunity was utilised for ascertaining the fungus flora there, by sending a series of cultural media to be inoculated there on the spot with fresh bladder material from haematuria animals destroyed for the purpose. Two animals became available, and when the tubes were

received back at Mukteswar, the writer was struck by finding pure growths of a yellowish-green fungus in more than one tube from each case. It was remarkable that these fungus growths resembled closely those that had already been encountered in Mukteswar materials.

Similarly through another officer, who was visiting villages in the interior of Kulu Valley in connection with his own investigation upon the warble fly (Hypoderma lineatum), samples of haematuria urine were secured. These again provided cultures of the yellowish green *Aspergillus*, and numerous perithecia were detected in the urine. (Plate 8. Fig.II).

The same type of *Aspergillus* was thus found to be associated with cases of haematuria in such widely separated parts of India, as the Kulu Valley in the Punjab, the Kumaun hills in the United Provinces, and the Darjeeling district in Bengal. It may be explained here that this type of *Aspergillus* does not appear to have been encountered ever before in India.

In an endeavour to ascertain the source of the *Aspergillus* associated with haematuria cases, and to trace whether any special vegetation of the localities were implicated, specimens of the bracken fern, and of the common types of lichens obtaining there were submitted to cultural examination. Again an *Aspergill-*

us, indistinguishable in chromogenic and growth characters from the cultures recovered from diseased animals, was obtained from each of the above-mentioned vegetation. In order to ascertain the identity of the lichens, specimens were forwarded to the British Museum of Natural History, and the following report was received:-

"Identified as

- (1) Usnea subsordida Stirt.
- (2) Parmelia reticulata Tayl.
- (3) Parmelia Camtschadalis Eschw.

All of these have Protococcus algae as gonidia. Moulds such as Mucor and Aspergillus will only appear on dead lichens in a damp atmosphere, just as with any other organic matter".

Since glycerine was found to be a medium which supported the growth of an Aspergillus to the exclusion of other organisms and bacteria, it was thought advisable to employ it for effecting the original isolations of this fungus from diseased animals. Before employing it for the purpose it was thought advisable therefore to determine the highest concentration in which it could be used to the most advantage.

The urinary bladder of an Ayrshire Cow No.4/27, a natural case of chronic haematuria, which died on 18.11.37, was collected immediately on death. The

organ was removed from the body intact, with precautions to prevent any contamination taking place. The organ was washed in several changes of sterile normal saline. Then small portions of the bladder with obvious lesions on the mucous membrane were cut out and sowed into tubes, containing different concentrations of glycerine in distilled water, which were previously carefully sterilised. The tubes were kept at the room temperature. 'Cottony' growths of fungus mycelia appeared and were visible in about three weeks. The period could be reduced by incubating the culture, but the tissue would undergo a rapid maceration. Growths in all the tubes were alike and indistinguishable, excepting that in the smaller concentrations of glycerine the fungus appeared to grow better and quicker than in higher concentrations. The fungus apparently would not grow above the concentration of 30%. This method can also be used for recovering Aspergillus sp. from vegetable matter. The result obtained with the diseased bladder tissue is tabulated below, and uninoculated tubes of glycerine were maintained under the same conditions as controls. The control tubes remained sterile throughout:-



Percentage of glycerine	Uninoculated control	Inoculated with bladder lesion
1. 20%	No fungus growth	Fungus growth
2. 20%	" " "	" "
3. 25%	" " "	" "
4. 25%	" " "	" "
5. 30%	" " "	" "
6. 30%	" " "	No growth
7. 40%	" " "	" "
8. 50%	" " "	" "

} Equal  
growth

It was found later that highly concentrated solutions (25% - 35%) of sugar (glucose, sucrose) could be employed for the purpose of isolating *Aspergilli* equally well.

#### Pure cultures.

Of the methods employed for obtaining pure cultures, those of decimal dilutions and pour plate, or of

multiple strokes have already been referred to. After a sufficient acquaintance with the cultures of the haematuria *Aspergillus* was obtained, it was found that by plating out the cultures one could without difficulty judge correctly whether the cultures were pure or contaminated. The growth characters of the pure colonies were so invariable and typical, and the effect of any extraneous contaminations so clearly manifest that the plating on the standard medium alone, was sufficient for ensuring the purity of cultures. Besides it was found that the main risk of any contamination taking place existed only at the time of the initial plating out, because once the culture was well-established on the medium contaminants had practically no opportunity of settling or thriving on the cultures.

It is however necessary to add that in special cases where critical studies were indicated, or the possibility of any lurking doubts had to be eliminated, the following procedure was adopted. No special apparatus or micromanipulator was used. The procedure approximated well nigh to the ideal of monospore culture. First as little as possible of the conidial 'powder' was transferred on the tip of a platinum needle to a tube of 10 c.c. of sterile water. The tube was then briskly rotated between the palms of the hands with a view to effect the separation of any conidia adherant to each other. A loopful of the dilut-

ion was transferred to a tube of melted agar medium, which had been brought down to an optimum temperature judged by the hand. The agar tube was briskly rotated and after a loopful from this had<sup>been</sup> transferred to a second tube, it was plated out carefully and rapidly. A loopful from the second agar tube was transferred to a third agar tube, and the second tube plated out. The procedure was repeated with two more tubes, and the original watery dilution was discarded. The plates were kept at the room temperature under an inverted belljar, and observed under the microscope after twenty-four hours. The medium formed a very thin and transparent film, and on inversion, a commencing colony or a single germinating spore, well-separated from any neighbours was marked off and carefully removed with a hot platinum loop together with a liberal amount of the surrounding substratum. This was placed on a sterile slide kept ready. Observations were continued with the progress of the growth. A precaution which was found to be important was that the plates were not to be incubated, as otherwise the growth was too rapid for any fruitful observations to be conducted at twenty-four hour intervals.

With a view to obtaining a preliminary idea as to how the *Aspergillus* cultures obtained from different sources behaved towards the different sugars, a series of fermentation reactions were carried out with eight strains. The results are given below:-

16.6.38.

Culture strain-	Glucose	Sucrose	Dulcitol	Mannite	Maltose	Dextrine	Salicine	Date
1. Fern	-	+	-	-	+	+	-	20.6
	-	+	-	-	+	+	-	25.6
	-	$\frac{1}{s}$	-	-	$\frac{1}{s}$	$\frac{1}{s}$	-	29.6
2. "Gaiety"	-	+	-	-	+	+	-	20.6
	-	+	-	-	+	+	-	25.6
	-	+	-	-	+	+	-	29.6
3. Hill Bull 459	-	+	-	-	+	+	-	20.6
	-	+	-	-	+	+	-	25.6
	-	$\frac{1}{s}$	-	-	+	-	-	29.6
4. Cow 4/26	-	+	-	-	+	-	-	20.6
	-	+	-	-	+	+	-	25.6
	-	$\frac{1}{s}$	-	-	+	$\frac{1}{s}$	-	29.6
5. Rabbit 9	-	-	-	-	+	+	-	20.6
	-	-	-	-	+	+	-	25.6
	-	-	-	-	+	+	-	29.6
6. Heifer Calf 38	-	+	-	-	+	-	-	20.6
	-	+	-	-	$\frac{1}{s}$	-	-	25.6
	-	+	-	-	$\frac{1}{s}$	-	-	29.6



16.6.38

Culture strain-	Glucose	Sucrose	Dulcitol	Mannite	Maltose	Dextrine	Salicine	Date
7. Hill Bull 143	-	+	-	-	+	-	-	20.6
	-	+	-	-	+	-	-	25.6
	-	+	-	-	-	-	-	29.6
8. Lichen	-	+	-	-	-	+	-	20.6
	-	+	-	-	-	+	-	25.6
	-	+	-	-	-	+	-	29.6

Notation used above:

Acid and gas +

Acid only +

Slightly acid  $\frac{+}{s}$ Trace of acidity  $\frac{+}{Tr.}$ Remarks.

The fermentation tubes were observed at intervals of four to five days after the initial inoculation. The table shows that the metabolism of the cultures as tested only with regard to acid and gas production. The haematuria culture recovered through an experimental rabbit was the only one that produced any appreciable amounts of gas. Varying degrees of acidity were observed on sucrose, maltose and dextrine by all the cultures tested. On the other sugars no acidity was seen on the days of observation. The reaction in glucose was rather peculiar. It is known that in aspergillar cultures, acidity results in the earliest stages of growth and that a balancing of acidity and alkalinity goes on, depending upon the period for which the growth is allowed to proceed. The absence of acidity in some sugars does not necessarily imply a total failure to produce it, but perhaps only a rapid changing over in pH.

Culture on Natural Animal Substrates.

In the course of cultural examination that were carried out with the object of diagnosis and pathological studies, certain interesting results were obtained and are worthy of mention.

Jugular blood.

Large quantities of blood from clinical cases of haematuria were bled directly into sterile flasks from the jugular vein by the use of sterile canula. Full attention was given to the observance of precautions against contamination. The flasks were immediately sealed and maintained undisturbed at the room temperature. A striking picture was produced after about a fortnight, and for some time afterwards the peculiar cherry-sized nodular elevations that appeared on the blood clot continued to enlarge in size. Subsequently minute white colonies of fungus growth started on the sides of the vessel in proximity of the upper surface of the contained fluid. (Plate 48). Microscopic examination at a later date confirmed the nature of these nodules to be aspergillar. A careful and detailed search in the literature revealed that the occurrence of similar growths was already recognised as a problem in tinned condensed milk. The causative agent in this case also was an aspergillus



Jugular blood from a natural case of haematuria was bled directly into this flask with a sterile canula. Elaborate sterile precautions were observed. The flask was sealed immediately after bleeding, and maintained undisturbed at the room temperature. Cherry-sized nodular elevations appeared on the clot, followed by minute radiating fungal colonies on the sides of the vessel. The 'buttons' were found to be due to Aspergillus flavus series. This finding was confirmed in two other cases, subjected to the same technique. The only <sup>traceable</sup> record of similar growths refers to an aspergillar infection of condensed milk. (Rogers, Dahlberg and Evans, 1920, "Cause and Control of "Buttons" in Sweetened Condensed Milk", Jl. Dairy Sc., 3, 122.).

(A.repens). The occurrence of an aspergillus in the jugular blood was confirmed in two other cases.

#### Diseased bladder.

On one occasion the urinary bladder, collected aseptically from a haematuria case was cut into halves and maintained in sterile petri plates in the refrigerator with the mucous layer lying above. When the plates were taken out it was surprising to notice that the white downy fungus growths that had taken place on the bladder surface were restricted clearly to the areas containing the bladder lesions, while the smooth and apparently healthy areas were free from any fungus growth. When the plates were maintained at the room temperature for about two days, the characteristic pigmentation of the haematuria aspergillus manifested itself.

#### Healthy bladder.

With a view to form a general idea of the invasive powers of the haematuria aspergillus, as well as for understanding some of the morphological forms exhibited in animal tissues, cultures were studied on healthy bladders. Healthy bladders were collected with sterile precautions from perfectly healthy animals, which were destroyed for the supply of meat for media preparation. The specimens of bladders



were washed after collection, in several changes of sterile saline and absolute alcohol. When the alcohol had evaporated, a small quantity of an old dried culture of the fungus was deposited on the mucous membrane and the opening of the organ was tied up. In order to ensure the maximum passivity of the culture and marked results, the dried inoculum was specially chosen. The bladder was placed in a sterile container in the refrigerator and maintained there for some time. The specimens of bladders were removed at various intervals, and sections were cut and stained, when the fungus was found to have penetrated to considerable depths into the bladder wall, depending upon the time elapsing after the inoculation. In the living body, the invasive capacity of the fungus would naturally be of a different order, due to the higher body temperature and other factors. The process of actual budding of the conidia was observed for a short while.

#### Rabbit's Ears.

The largest ear vein was picked and carefully sutured at two ends. A small quantity of the highly pigmented aspergillus culture was placed inside the vessel with the help of an injection needle. After varying periods of time, the portion of the affected vein was excised from the different animals. Sections were cut and stained, but it was difficult to detect

more than very few of the conidia in the sections. It was not possible to judge whether the organisms had passed through the vessel walls, or were taken away by the collateral capillaries. Experience gained from these preliminary studies were of considerable value later.

#### Optimum Temperature Studies.

With a view to determine the pathogenicity of strains of *Aspergilli*, authorities have employed optimum temperature studies as one of the major criteria. Half a dozen of the cultures from haematuria cases were therefore taken at random and their optimum temperature of growth determined. First each one of the cultures as subcultivated, and when forty-eight hours old, each one was subinoculated into three tubes of Sabouraud's medium. These were then incubated at different temperatures, the incubators being maintained at 22°, 30°, and 37°C.. It was found that all the six cultures tested showed the same character and rapidity of growth at each incubation temperature. Besides individual cultures showed better and more luxuriant growths at the higher temperatures and 37°C. was found to be the optimum for each culture tested. The difference in the relative rates of growth of each culture at varying temperatures was directly proportional to the temperature from the very commencement, and this

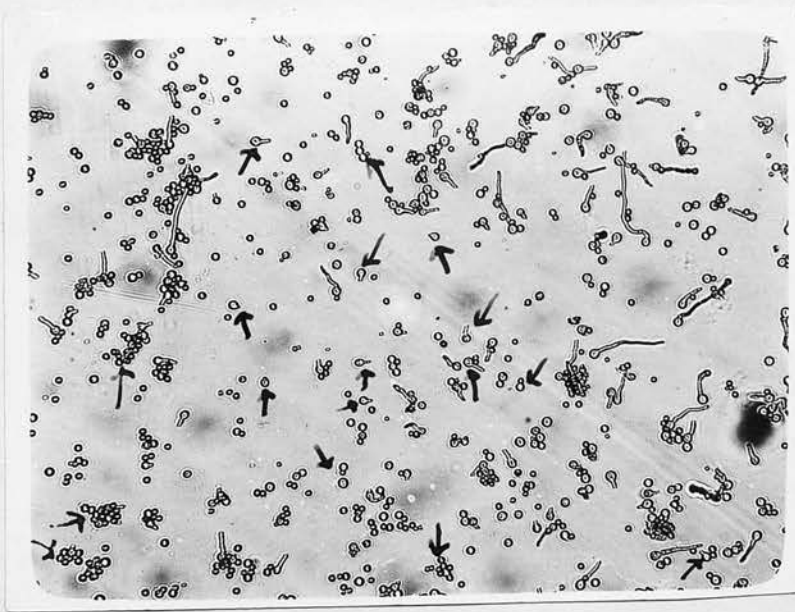
Plate No.49

Fig.I. Germination of conidia. Early stage. The majority of conidia are still unbudded, some are just starting, others have progressed to some extent. Curiously, under conditions in the urine or in the tissue a considerable number of conidia at the early stage of commenced budding are present in large numbers rather than in varying numbers of differently developed conidia. Examination of haematuria urine often reveals incompletely budded conidia. Sometimes more than one bud is extruded, suggesting pseudopodial action of protozoa.

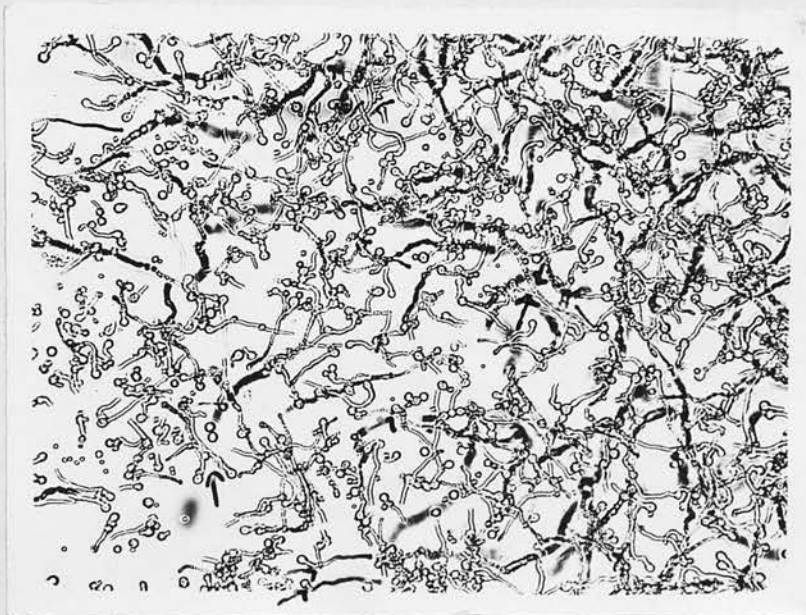


Fig.II. The same as above. At a later stage. When germ tubes of this type are interspersed among fibroblastic tissue, both young and old, in protracted lesions, as have often been found in the kidney, the state of confusion that is likely is imaginable.

relationship was maintained from day to day till the cultures ceased to grow any further. The highest temperature that would support growth was not determined. In passing however, it may be stated that a preliminary test upon the resistance of the cultures to high temperatures was carried out by subjecting them to conditions under which bacterial cultures are successfully autoclaved. When these cultures were ground and emulsified, healthy rabbits were inoculated with them. Intraperitoneally, and by injection into the lung, multiple abscesses were set up in some of the internal organs. When sections were cut and stained, it was found that the lesions were definitely due to fungal activity. The tenacity of the cultures will be discussed elsewhere.



VII. (b) THE PARASITE FROM DIFFERENT SOURCES  
IN CULTURES.

As already explained, attempts were first made to isolate the fungus flora from as many haematuria cattle, originating from as well separated areas, as possible. Different pure isolations thus became available. These were maintained to see whether with a sufficiently comprehensive number of isolations having accumulated, cultures of significance would not automatically sort themselves out as the commonest or the invariable strains. Any medium that happened to be readily at hand, whether liquid or solid, was utilised, the object being to effect successful pure isolations only. In the absence of any clue as to the nature of the fungus to be specially sought for, no standard medium was used. Twenty-three cultures thus resulted from eight clinical cases of haematuria then available at Mukteswar. The isolations were repeated, and while for instance, the same type appeared from an animal more than once, additional cultures of what superficially appeared to be different were obtained, and this complicated the issue. To take extreme cases, while one animal repeatedly produced a yellowish green culture, another produced as many as four cultures then described as (i) slightly yellowish white downy growth, (ii) greenish yellow powdery

growth, (iii) dirty brown granular scanty growth, and (iv) slightly black downy exuberant growth. The last mentioned was later found to be Aspergillus niger, while the first three were, to all intents and purposes, cultures of the same Aspergillus sp.. The considerable variation in growth characters and pigmentation shown by the different isolations became perplexing. Sooner or later most cultures appeared to pass through a greenish or a greenish yellow colour. Microscopic examination of the morphology was found to be equally complicated. Characteristic aspergillar heads were present, but no uniform shape or size of the specific structures could be fixed for the cultures, to enable an opinion being formed of their identity. The cultures were sometimes white and downy, at others definitely but only mildly pigmented green or yellowish green, at others again they tended to become powdery and brown. It became difficult at that stage to decide whether one or more species of Aspergillus was involved, and whether the cultures belonged to one or several of the groups - A.glaucus, A.flavus-oryzae, A.tamarii and A.versicolor. In the conidiophores, various gradations in shape and size of the vesicle from a perfectly globular to a dome-shaped semi-circular head, or a columnar elongated head were present. The foot-cells were sometimes not recognisable. What was more complicating was the presence of

atypical heads. The vesicles were sometimes considerably abbreviated, or even appeared to be absent. The sterigmata were generally in one series, but there were heads with distinct secondary sterigmata or conidiophores. Were the cultures then a Sterigmocystis, or a monoverticillate Penicillium? Comparatively large and globular masses of conidia, apparently adherent together were often present. Was there a Gliocladium? The characteristic aspergillar heads were there, though the heads were somewhat dissimilar, but subcultures after repeated plating and decimal dilutions, left no possibility of doubt about the purity of the isolations. The finding of abnormal conidial heads with a green aspergillus in this case remained inexplicable. The possibility that organisms, belonging to other genera, related to the Aspergillus and Penicillium (Penicillopsis, Gliocladium, Hormodendron, Citromyces etc), might be involved, was examined, but the confusion continued. The presence of contaminating fungi of the bacteriological laboratory, including A.niger, Mucor, Penicillium, Rhizopus made the situation worse. The burden of maintaining subcultures was becoming more and more time consuming, and the fear of contaminating one culture with another was looming large. The difficulty of making any satisfactory study of so many cultures at one time, in the absence of some clear indication of their botanical

relationship, was keenly felt, and an opportunity for the drastic curtailment of the cultures presented itself. The writer was transferred from the Headship of the Pathology Dept. to organise the newly built Protozoological and Entomological Dept. of the Institute. Before moving to the newly built laboratory buildings, it was considered advisable to discard all the old cultures. Clinical cases were still present. If a mould-fungus was the causative agent of haematuria, the cultures should repeat themselves constantly and invariably. If they did not repeat themselves it was just as well not to waste any further time over them.

At this time a valuable clue suddenly presented itself. Tubes of culture media, inoculated on the spot directly with fresh bladder material from two haematuria animals specially destroyed for the purpose were received back from Kalimpong. On arrival at Mukteswar, more than one tube from each of these cases were found to contain practically pure growths of a yellowish green *Aspergillus*, looking extraordinarily like the cultures which had been encountered so many times previously in Mukteswar cases. Those two cultures were subcultivated on several media, and appeared to be identical in the colony characters, pigment production and in minute morphology. In order to have the opinion of an expert as to whether the two



cultures were completely identical, they were forwarded to the Imperial Mycologist (Mr L.D. Galloway) at New Delhi. The report received from him reads as follows:- "The two cultures have been given a preliminary examination. They appear to be identical, and a species of *Aspergillus* which does not fit in with the described species but comes fairly close to Asp. tamarii. If I can get a closer identification, I shall let you know". In a later report it was stated that the two strains were handed over to a friend in London, who placed them in *Aspergillus glaucus* group, somewhere between *Aspergillus ruber* and *A. scheelei*.

Later another culture isolated directly from the bladder tissue of a cow (Cross-Ayrshire No.4/27) at Mukteswar in March, 1938 was forwarded to the same office then held by another officer, who reported:- "The fungus is a species of *Aspergillus* belonging to the group Versicolor, and is quite distinct from any previous cultures you have sent. The group, Versicolor contains pathogenic forms". Later still two further cultures were similarly isolated from the bladder and heart blood of two other cases sent on 17-6-38. A third officer, (Mr G. Watts Padwick) who held the post then reported: "Culture No.1 appears to be of the series flavus of the group *A. flavus-oryzae*. Culture No.2 contains the same fungus but also a Peni-

cillium which we have not attempted to identify".

On his way to Europe, the present writer forwarded a set of eleven cultures of Aspergillus sp., recovered from haematuria cases and from the bracken fern and lichen, to Mr. George Smith at the London School of Tropical Medicine, for favour of looking after the cultures till the writer arrived in London. A second set of the same cultures were brought in person with a view to make a more detailed study of the cultures with Mr. Smith, who was known to be personally interested in the genus Aspergillus. As the writer commenced his studies at Edinburgh and did not have the opportunity of returning to London to undertake the proposed study with Mr. Smith, he enquired if the latter had formed any opinion on the series of cultures that were sent to him from India. The reply reads: "The cultures which you sent to me in July were all strains of Aspergillus flavus Link. As I did not hear from you again after your visit in September I concluded that you had no further interest in the cultures and so scrapped them all before Christmas". It may be added here that the cultures sent to Mr. Smith included the isolations already examined and reported upon by the Mycologists mentioned above, besides those that were recovered from lichen, and bracken, and a few other cultures from haematuria animals.

It may now be mentioned that the writer employed

Plate No. 50

Fig.I. Culture (old) on sterile bread. Note the loss of pigment in the secondary colonies, a property frequently exhibited by most strains. These do not appear to be true mutations. Note also the dispersion of 'powdery mould' to distances away from the medium. Brown bread produces more luxuriant and rapid growth than white bread. The subtle differences in the quality and quantity of nutriment in different media are readily brought out by the character of fungal growths.



Fig.II. Old culture on slices of carrot. Note the differences in the character of growths on the three slices, also the dispersed powdery conidial masses.

for his morphological and physiological studies, the following twelve cultures of the Aspergillus sp., with a view to their classification and identification. It will be noted that with the exception of one culture which was recovered at Edinburgh, the rest were isolated after the writer's transfer to his new laboratory, and that the cultures refer to specimens from Kalimpong, the Kulu Valley, the Nilgiris and Mukteswar:-

- (1) Isolated directly from the bladder of a naturally affected Cross-Ayrshire Cow "Gaiety" at Kalimpong, destroyed for the purpose of obtaining cultural material in October 1936.
- (2) Isolated directly from the bladder of a naturally affected Cross-Ayrshire Cow "Susan" at Kalimpong, destroyed for the purpose of obtaining cultural material in October 1936.
- (3) Isolated from the jugular blood of Cow 4/26 on 16-6-38.
- (4) Isolated from the same animal from bladder tissue on 16-6-38.
- (5) Isolated from the bloody urine of a Cow in the Kulu Valley.
- (6) Isolated from the heart blood of an experimental healthy calf, (H.C.38) which died of too frequent administrations subcutaneously of a strain of Aspergillus isolated from a natural case.
- (7) Isolated from the bladder tissue of a case of



Plate No. 51

Fig.I.

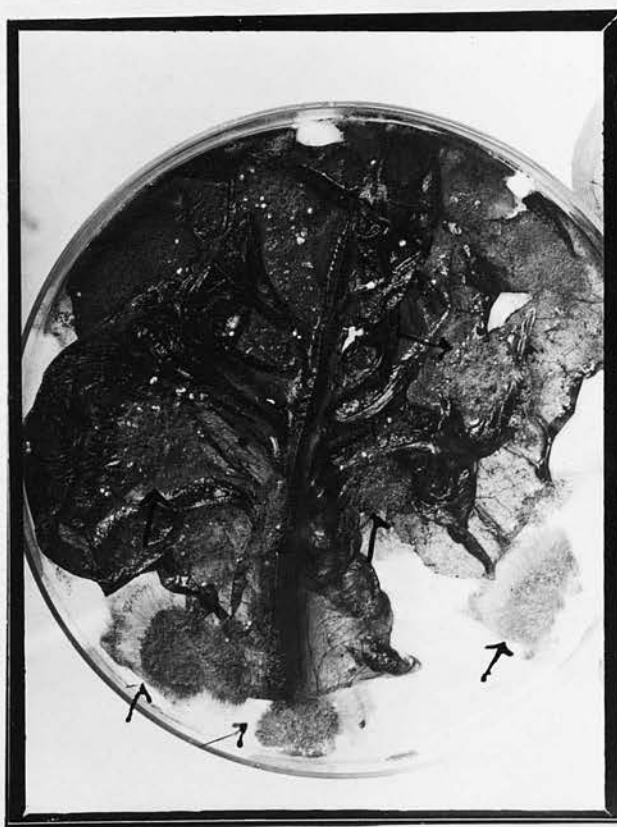


Fig.II.

Culture on sterile leaves. Fig.I.represents the character of growth on lettuce and Fig.II. that on cabbage. Certain areas are seen to be matted with the growth and certain other areas show how the leaves have been eaten into by the mould. Fig.II.shows at the bottom colonies divided into a darker central zone and a peripheral lighter zone.

successful experimental transmission (H.B.143) effected with subcutaneous injections of haematuria urinary sediments, on 26-5-36.

(8) Isolated from a clinical case (H.B.549) at one of the Outkraals of the Mukteswar Institute.

(9) Isolated from a rabbit (No.9) inoculated intravenously with material from a haematuria cow on 30-4-38.

(10) Isolated from bracken fern at Mukteswar.

(11) Isolated from lichen at Mukteswar.

(12) Isolated at Edinburgh from a specimen of diseased bladder tissue received in 50% sterile glycerine from the Wellington Dairy Farm in the Nilgiri Hills in South India, in December 1938.

#### VII. (c) MYCOLOGICAL STUDIES.

##### Cultivation on various media.

The twelve cultures listed above were planted out in culture tubes and in petri plates containing a variety of solid media. Even at the most superficial view of the cultures one could see that they produced the same invariable colony characters, and passed through similar grades of colour changes. The close relationship of the cultures, and the suspicion that the whole set represented an identical organism emerged from the very outset. Before attempting to identi-

fy and classify the cultures, it was necessary to know the range of their variation under each set of circumstances. Different sugars were substituted in each medium and the effect studied. It was found that when the cultures were young and in the very commencing stages of growth, they appeared to be whitish and practically unpigmented. Later as the growth developed, pigmentation gradually manifested itself and the colony colour became deeper to some shade of green. Ridgway's colour standards were used to compare the cultures from stage to stage. The vegetative mycelium did not appear to produce any appreciable pigment, and pigment production appeared to be restricted exclusively to the conidia and conidial heads. It was noteworthy that practically identical amounts of transpiration water and calcium oxalate crystals were produced by each member of this collection of cultures. As far as sclerotia were concerned, there appeared to be some minor differences in the amounts of these produced and in the rates at which they appeared. The maximum amounts of the oxalate crystals appeared on the pellicle cultures on liquid media, such as horse digest medium (Plate 65) or glucose broth. There was one other type of crystals noticed in these cultures, and they appeared to be those of earthy phosphates. It may be remembered that these two types of crystals

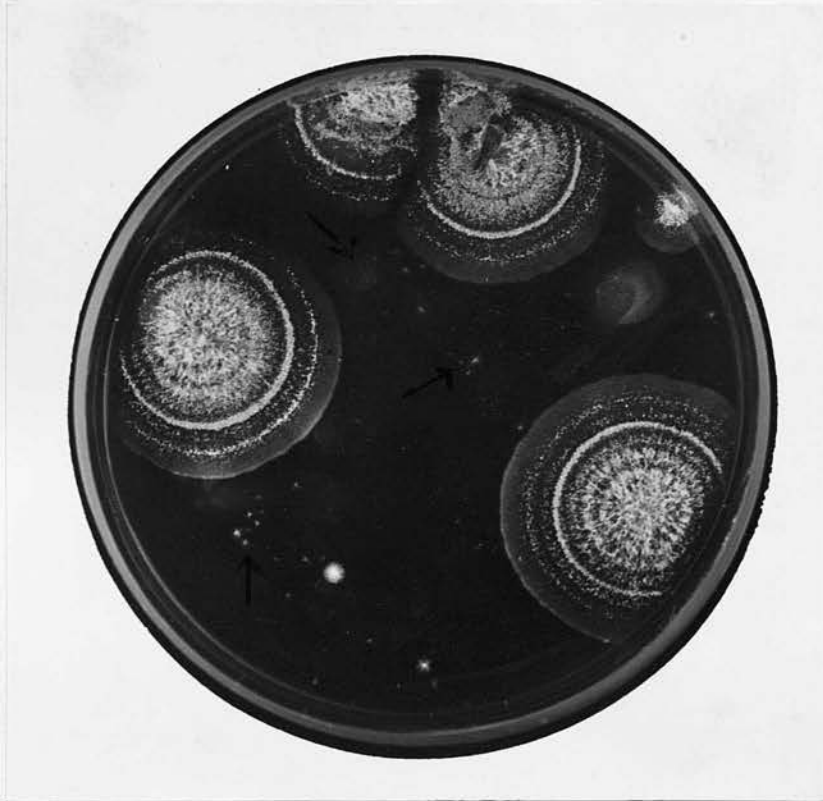
occur in the affected bladder tissue and sometimes also in the urine of natural cases of haematuria. More uncertain than the sclerotia were the variations in the brownish pigmentation that appeared on the reverse side of petri plates of different cultures. It seemed possible that these brownish patches were actually connected with the sclerotia.

#### Cultures on different Sugars.

Of all the sugars tested, it was found that the cultures produced the least growth on lactose, taking a longer time to attain the developmental characters comparable to those on other sugars. Glucose produced the most luxuriant growths while saccharose produced almost equally good results.

Cultivated on the routine Lemco Agar, with one or the other sugar being used in the composition, it was found that the cultures attained to a height of about  $5/6$  m.m. above the surface of the medium. When glucose was used in the medium, the colour produced was somewhat different from those exhibited on other sugars. Glucose gave a Mignonette green (Ridgway - 25 YG - Yi Plate XXXI). Saccharose yielded a somewhat similar growth but there was nothing very particular to be noted in the cultures on this sugar. The cultures on Maltose-Lemco-Agar were different, with the centre of the colonies being nodular and of a darker colour



Plate No. 52

Petri-culture of the organism showing typical zonation. Looking from the centre the thick ring, situated at about the middle of the radius of the culture, represents coremia production. The nature of the zonation does not appreciably change if one solid medium is substituted for another. Note the minute colonies which have resulted from dispersion.

than the peripheral zone. The colour in the centre approximated to sea foam yellow (25 YG - Yf Plate XXXI), and the periphery was distinctly cottony and fluffy. On Lactose-Lemco-Agar the colony size averaged that of a sixpence. The growths were the least in amount on Lactose, and to start with, no pigmentation was discerned. Later however, cultures on it developed the identical and complete pigment production as noted on the other sugars.

#### Cultures on Czapek-Dox-Agar.

This medium has been recommended by previous workers as the best standard medium upon which the comparative and systematic studies of *Aspergilli* should be carried out. In order that the results of various workers may be comparable, the possibilities of pleomorphism to which this particular genus is extremely liable, should be eliminated by the use of synthetic standard media, such as Czapek medium.

In order to study the comparative rate of growth of the different cultures, it is essential that the same amount of inoculum be used in each culture tube or plate of medium. The colony characters are best studied in petri plates, but cultures have been simultaneously studied in tubes. They were usually incubated at 37°C., which had already been found to be optimum for the cultures. When the cultures were

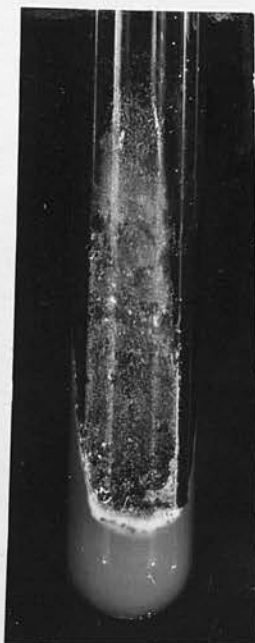
Plate No. 53

Fig.I. Culture on Czapek's medium. The colour reactions are very clearly brought out on this standard medium. The colour varies around yellowish-green. Drops of transpiration water are produced early, followed by encrustations of crystals and later the occurrence of depigmented secondary colonies. The different strains which have been recovered from the bladder tissue, jugular blood, urine of different haematuria animals in different parts of India, have been found to conform closely to each other in pathogenic action, colour reaction and other biological behaviour.



Fig.II. Culture on sterile slices of beet. The *Aspergillus* has produced a mat of growth on the medium as well as at the periphery. The organism grows readily on most vegetable and animal substrate, but perithecia are only very rarely produced by chance.

maintained at the room temperature, or at lower incubator temperatures, the most important effect noticed was that the rate of growth was substantially delayed. Alterations in the incubator temperature did not appear to produce any significant changes in the morphological characters of the various *Aspergillar* structural forms. Observations on the colony characters were started after twenty-four hours and continued at the same interval over a sufficiently long period.

After twenty-four hours of inoculation, the colonies were found to have started their vegetative growth from the central inoculum but nothing noteworthy attracted attention. After another twenty-four hours the appearance of the cultures was somewhat more marked, but still there was nothing worthy of description. At the end of seventy-two hours after inoculation, the cultural features were more clearly exhibited. The centre of the cultures was raised and a distinct white margin of appreciable width was clearly seen. The cultures were generally velvety. The pigment produced by each culture was exactly identical, as far as careful naked eye examinations could determine. On comparison with Ridgway's standard, the pigment conformed to oil-green (25 TG - YK Plate XXI). The growths apparently tended to become floccose. The medium itself retained its natural colour and no tend-



ency to any discolouration was noticed. Crystals started to appear. The next day the above growth characters were more accentuated. On the fifth day the cultures were decidedly greener. The average colonies varied from 1 c.m. to 3 c.m. but occasional single colonies were as large as 6 c.m.. Three zones could be distinguished e.g. central, ring of short coremia, and peripheral zones (Plate 52). The colour of the cultures varied from jade green to Carro green (27 GY - K, XXI. to 27 GY - K, V). The water of transpiration appeared to be at its maximum. On the reverse side the centre of the larger colonies stood out due to a brownish umbilicated crust. Whitish mycelial strands were seen radiating out from the brown centres. On removing the crusty brown material, it was examined under the microscope and found to be sclerotia.

In the next day or two there were few changes to be noticed in the cultures. The water of transpiration began to dry off about the sixth day, and practically half the cultures did not show any water on the seventh day. In some cultures this water persisted for another day or two. On the seventh day the pigment varied from Kronberg's green to jade green (25" YG - YK to 27"G - YK, Plate XXI. Ridgway). On the eighth day, most Czapek plates showed white crystals

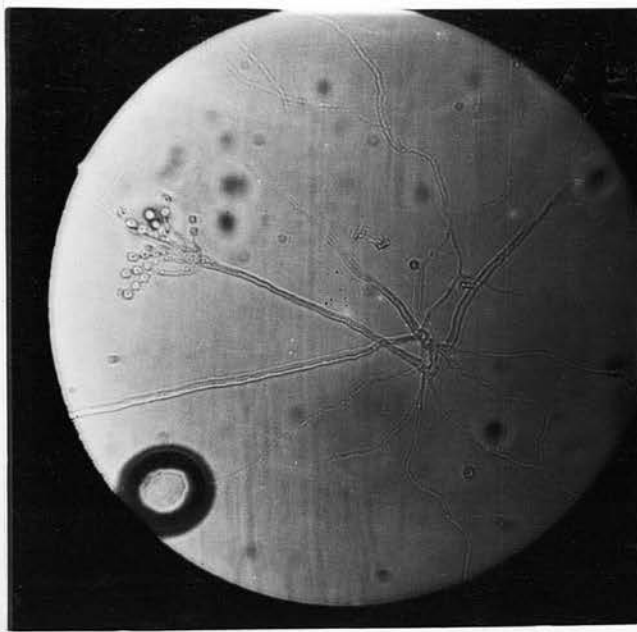
Plate No.54

Fig.I. Culture. An individual thallus, showing the vegetative and the reproductive parts. The conidiophore is not quite typical, the vesicle being ill-developed, though the foot-cell is recognisable.

Atypical heads are often produced in the animal body, as also in culture, sometimes making the confusion exaggerated, and the possibility of the organism being other than *Aspergillus* quite marked.

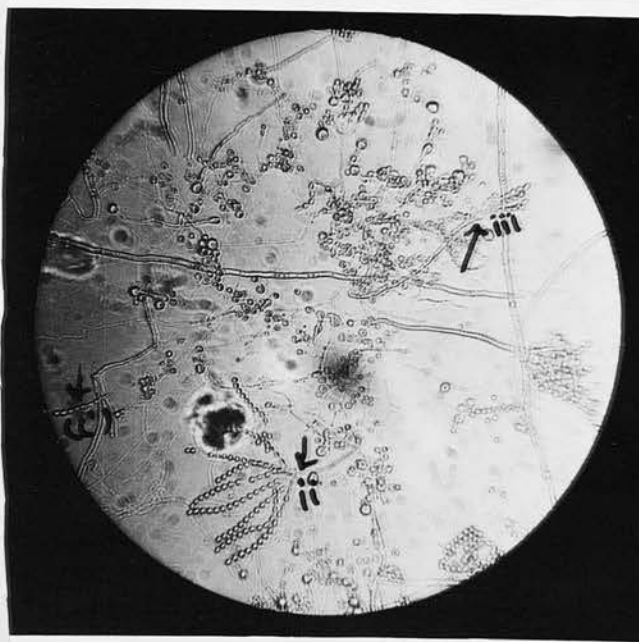


Fig.II. Culture. Shows several conidial heads, (i) a solitary conidial chain growing in place of the typical head, (ii) a very atypical head resembling greatly that of a *Penicillium*, (iii) a narrow head with three chains, recognisable vesicle but undeveloped foot-cell.

at the junction of the middle and peripheral zones of individual colonies. The crystals began to appear from the third day onwards. The colour of the colonies varied from Calla green to Cerro green (25 YG - Ym to 27 GY - m, Plate V.). As the growth became older, it was noticed that after a time when the colonies had reached a particular stage no further extension from side to side took place. The conidiophores however grew higher to a certain limit. It was found that a process of dispersion of conidia to considerable distances away from the foci of colony development, took place. Consequently with time a number of minute colonies began to appear in close and increasing succession in different parts of the plates. Besides it was observed that the tendency to any further change of colour of the cultures was practically non-existent as far as cultures on this medium were concerned. The cultures practically came to a standstill. As the moisture content was being gradually exhausted they became extremely powdery, and the powdery material itself was so loose that the slightest tilting of the plates would make it run down to the lowest level in plates. The cultures were kept under observation for long periods up to one year, but no perithecia developed, except in three accidental cases to be noted elsewhere. On the basis of the data

recorded by workers as being optimum for perithecia production in certain groups of *Aspergilli*, the concentration of sugar was raised and the cultures were incubated at 22°C. No perithecia however resulted with the haematuria cultures in the ordinary way.

Cultivated in tubes, the cultures covered the whole surface of Lemco medium prepared with glucose or saccharose, in about four days. Cinnamon brown drops of liquid appeared and showed a tendency to coalesce and flow down to the lower parts of the culture tube. This feature was not so marked on Czapek cultures. The drops were produced on nodular growths which were composed of closely aggregated mycelial strands. The surface of these nodules initially appeared to be white and irregular. Later these became rather hard and resilient, and were recognised as the typical sclerotia. With the progress of time these became brown.

Of the different solid media used, Czapek produced the greenest cultures, more colouration being evident in the thinner portion of the slant, though later the initially white colour in the lower and thicker parts of the slants assumed the characteristic depth of pigmentation. (Plate 53, Fig. I.). After six days of inoculation the cinnamon brown liquid drops were still present on Czapek. The medium con-



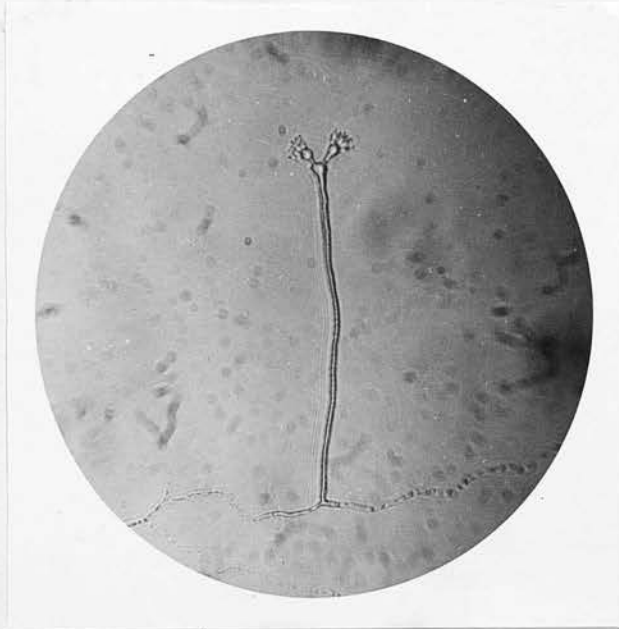
Plate No. 55

Fig.I. Hanging drop culture. Conidiophore, showing a-typical vesicle, giving rise to other secondary heads (2), but provided with the foot-cell.

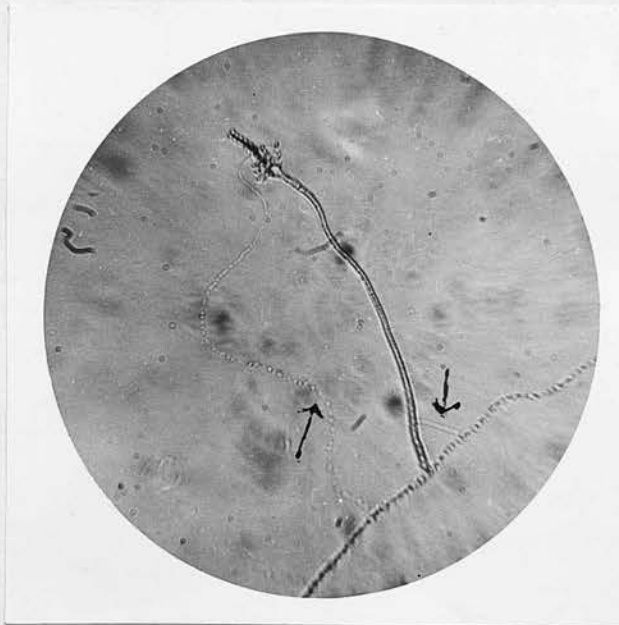


Fig.II. Same as above, showing abnormal development of the central conidial chain of the head, also an additional supporting bracket-like structure, apparently not described before.

taining lactose as the sugar, showed the least of the green tinge, and the pigment conformed to Ecrú-olive (21" OY - YI, Plate XXX.). If the growths on Czapek, and Lemco-Agar containing different sugars are arranged according to the intensity of colouration from green to Ecrú-olive, they could be arranged in the order Czapek with sucrose, Lemco-Agar with each of glucose, sucrose, maltose and lactose. As noticed on petri plates, the best growths were produced on glucose and saccharose, and the least on lactose. In regard to the sclerotia, which were rather hardened, tenacious, and resilient on pressure, they were produced in largest numbers in media containing saccharose. On the media containing maltose, glucose, and lactose, they were produced in gradually diminishing numbers in the same order in which these sugars are mentioned here. In colour the sclerotia on Czapek were more white than on any other sugar-containing medium. On Lactose-Agar slants, the growth of the fungus was most dense in the centre of the colony, and lower down towards the bottom and thicker portion of the slants, the cultures tended to be very fluffy and white.

#### Culture on Vegetable and Bread Substratum.

With a view to test the behaviour of the cultures with regard to the production of perithecia, several kinds of natural vegetable substratum were used.

Of these, boiled tea leaves, sterilised cabbage, lettuce, (Plate 51) potato slants, slices of beetroot, (Plate 53, Fig. II.) carrots, tomatoes, prunes and bread (Plate 50, Fig. I. & II.) were employed.

Regarding the details of bread cultures, it may be said that portions of both white and brown bread were sterilised in petri dishes. The same thickness of bread was taken. It was found that if the thickness of the bread was not uniform in the different plates a variation in results would naturally follow even with the same culture. This probably depended upon the available space in the petri plates left over for holding the oxygen supply and for occupation by the fungus growths. An interesting observation was that brown bread gave a more luxuriant growth to start with, and the developmental stages were passed more rapidly on this medium than on white bread. An extremely small inoculum was used and the same quantity was placed on both the varieties, but the brown bread was covered over the whole surface in a much shorter time. In brown bread the fungus penetrated into the depth of the bread and could be seen on the reverse <sup>comparatively</sup> side / soon after inoculation. The conidial heads appear to be restricted to the centre of the colonies. On the subsequent day, the colonies had not only extended in size but the conidial heads

Plate No. 56

Fig.I. Culture, showing columnar heads. The heads are usually globose, or dome shaped. The difference in the shape and character of the head appears to depend upon the rapidity of growth, the columnar forms being stimulated under particularly luxuriant conditions.



Fig.II. Culture on slide. Showing mycelia and a few conidiophores. The flower head in the centre illustrates a common form seen in smears from diseased bladders. Note how a hyphal tube in the centre of the field has become abnormally thickened, this being a frequent finding in affected animals. The thickened hypha resembles cotton fibres.



were present almost equally at the periphery as at the centre. After three days of growth the pigment produced conformed to Kronberg's green (25 YG - YK Plate XXXI.). The reverse of the plate showed at the periphery of the bread practically the same amount of pigment production as on the upper surface. The fungus spreading on the lower surface of the bread was inclined to be white and comparatively unpigmented in the central zones, as opposed to the periphery of the bread.

Turning to the white bread, the culture was much smaller on the fourth day, and restricted mainly to the central area of the upper surface. It had not penetrated through the thickness of the bread, nor could it be seen on the reverse. The colour that was produced was not so intense as that on the brown bread, it being some tint of lime green. When the cultures were examined the next day, the growths had extended themselves further and the colour changed to Mignonette green (25"YG - Yi, Plate XXXI.). The conidial heads preponderated in the central area and extended to the middle zone of the colonies. The peripheral zone of individual colonies continued to be cottony. The pigmentation was decidedly more developed than on the previous day. Slowly and gradually the whole bread was covered up with the felted

Plate No.57

Fig.I. Section of a piece of potato, invaded by the fungal culture. Artificially grown with a view to studying the inner details of the mycelium in different stages. This method obviates the difficulty of preventing smears of culture being washed away during staining processes, from glass slides. Note the black dots and vacuole-like structures along the length of hyphae. cf. Plate 39.

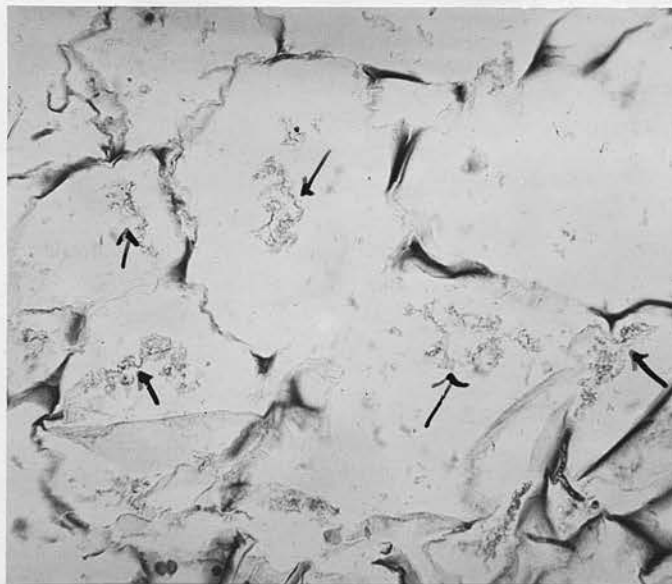


Fig.II. Same as above. From a deeper portion of the potato, with lesser oxygen supply. Note the difference in the character of individual fungal colonies from that shown above. The organism appears to have lost its power of elongation in the longitudinal direction, having become finer and more delicate, granular and rather ill-defined in its characters. The difficulty attendant upon its recognition in animal tissues, particularly in acutely haemorrhagic lesions, is obvious.

culture, the characters of the culture conforming more and more to that on brown bread. The cultures were maintained for a considerable time to see if any perithecia would be finally developed. It is known that bread forms a particularly favourable medium for stimulating the production of perithecia and that usually they appear within a week or a fortnight at the most.

Regarding the other vegetable media already mentioned, it is not necessary to go into any details. The cultures were carried out primarily with a view to studying perithecia production. The object was not achieved. It may however be added that the cultures were found to be able to grow readily on all the different vegetable media so far tried. The cultures enabled a general impression to be gained of the growth characters under natural conditions. It was found that the growths were influenced to a large extent by the degree of moisture conditions available. Cultures as exhibited on these vegetable media are illustrated in Plates 50, 51 and 53.

#### Cultures on Liquid Media.

A number of liquid media including glucose broth, 5% peptone, sugar solutions, tea infusion, glycerine, horse digest medium, were used. The different cultures were grown on these media in test tubes and in petri plates. The characters of the fungus growing on



Fig. I. Culture on glass slide, treated with collodion solution to prevent organisms being washed away, fixed in Fleming's solution and stained. Under high magnification, showing how only odd cells from the hyphal branches stand out, the other neighbouring parts being nearly invisible. Note the chains of 'streptococcus-like' fungus bodies.

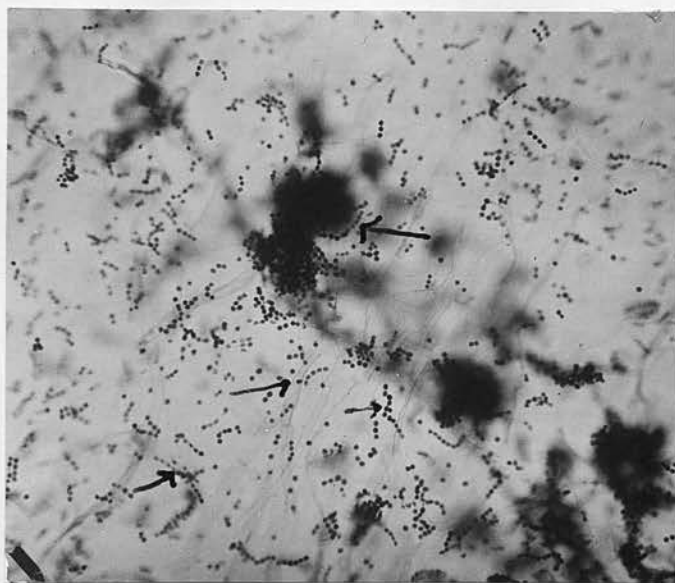


Fig. II. Same as above, to show a general view of the fungus in culture on a slide, containing the mycelia and chains and massive clumps of 'spores'. This is highly informative when studied side by side with preparations made from samples of haematuria urine, and with sections of the diseased bladder. cf. Detroye's micrococcus.



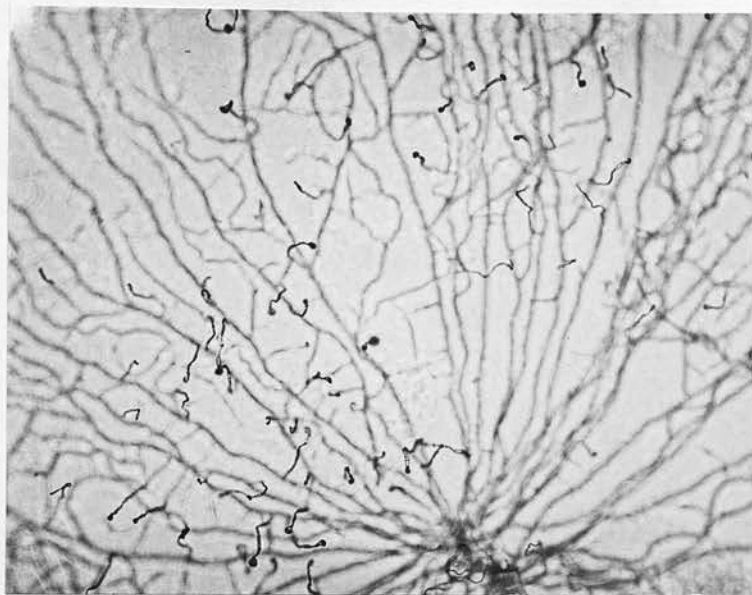


Fig I. Culture on slide, showing the commencement of the production of the dangling reproductive mycelium on the vegetative matrix. The resemblance of these to the like structures in tissue sections is very striking. X 75.



Fig.II. Same as above, after the full development of the conidiophores. X 165.

moist slides in petri plates can be examined directly under the microscope by uncovering the culture. Some of the atypical forms which are readily produced on glycerine, and concentrated sugar solutions are illustrated in Plates 54 & 55. On horse digest medium, a pellicle culture was obtained, and as this thickened hyphae were found to climb up the sides of the glass tube above the surface of the medium to about  $1\frac{1}{2}$  c.m.. Large amounts of calcium oxalate crystals are produced in these pellicle cultures. With the progress of time some of the surface growth deposits to the bottom, generally in flake-like masses. The rest of the medium however remains clear. In bottom cultures, in glucose broth or similar other media the character and progress of the growth are considerably changed.

#### Morphology of Cultures.

To summarise the growth characters of the cultures on various media:-

- (1) Rate of growth - moderate, greater in the incubator and on certain substratum, particularly on glucose agar media.
- (2) Colony colour and colour changes - uniform in all the cultures. Colony colour exhibited in the central zone. The edges of the growing colonies tend to remain somewhat colourless. Even in old cultures the edges are somewhat paler. On various media the green

shades verge from oil-green, Calla green, Mignonette green, Kronberg's green, jade green, Cerro green. On lactose, Ecrú-olive.

(3) Colour and colour changes on the reverse - reverse not generally pigmented, small circumscribed brownish pigments appear here and there, perhaps connected with sclerotia.

(4) Colour changes in the medium - colour production restricted to culture, no pigments diffuse into the medium.

(5) Texture of surface - growths generally compact over the whole surface, but small areas of loose growths appear in old cultures. The surface is plane, due to the aerial hyphae growing to about the same height. Occasionally wrinkling may be seen on prune extract agar. At the edges of giant cultures on Czapek-Dox-Agar in Erlenmeyer flasks, streamers of hyphae are frequently seen. In the latter, on the walls of the flask the streamers form numerous conidiophores radiating out to equal distances from the centre of individual colonies. This striking picture is produced by all the cultures.

(6) There is no characteristic odour of these cultures. Occasionally a generally mouldy odour is exhibited. Of all the media used the strongest odour was produced on sterile bread medium, and reminded one of

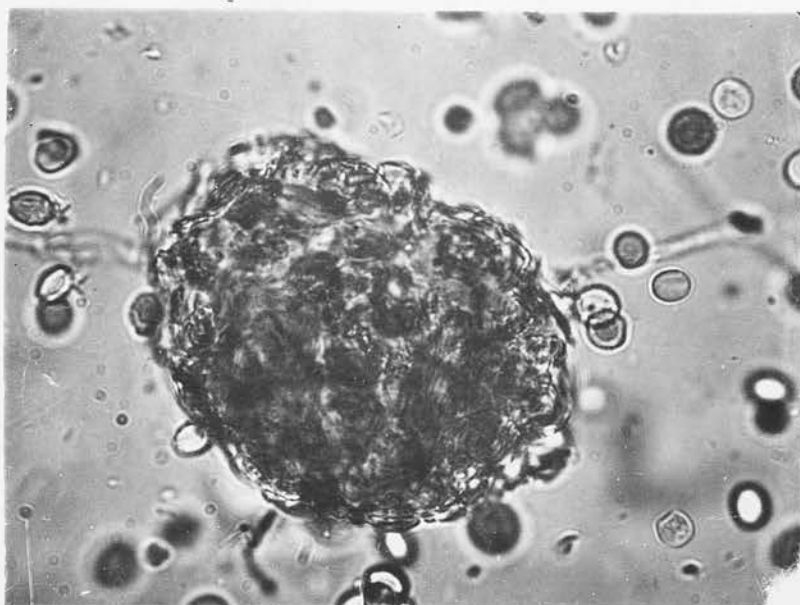
Plate No.60

Fig.I. Smear from old culture, showing single Perithecium, with a few grooved ascospores extruded from it. These have been detected in sections of diseased tissue particularly of the bladder. Refer to Plate 28 Fig.I.& II. X 850.

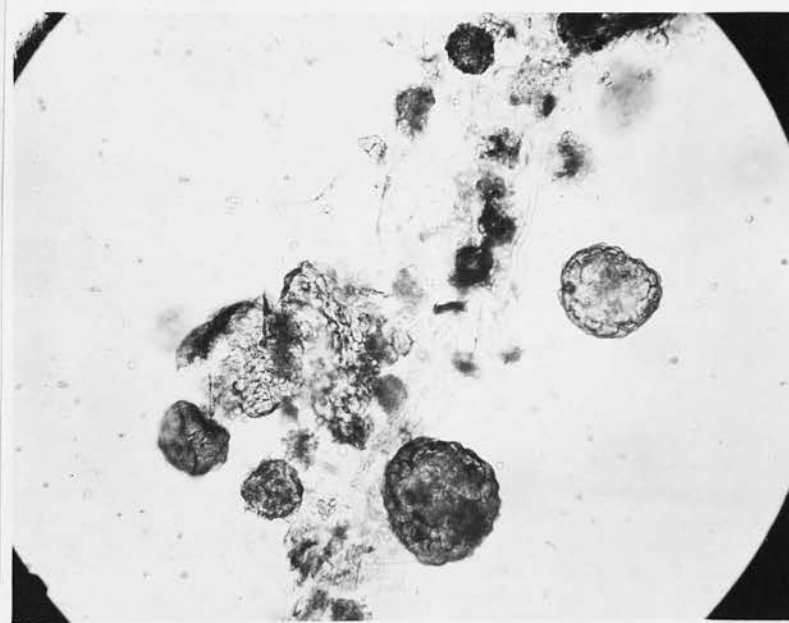


Fig.II. As above. Perithecia from an old culture. Note the different sizes and also the breaking and broken individuals. X.155.



fermented bread.

(7) Transpiration fluid appears on the aerial growth. The globules are shiny and transparent on Czapek. On other media sometimes brownish.

(8) Submerged hyphae - do not produce any pigment, are segmented, approximately of the same diameter as their neighbours immediately above them. Sometimes crystals seem to appear on them. Chlamydospores along lengths of hyphal tubes may be seen, under unfavourable conditions. (Plate 64, Fig. I).

(9) Among the fruiting bodies, intercalary and terminal chlamydospores and conidiophores are frequently present. Conidiophores appear comparatively early but a culture does not have its full complement for about four to seven days depending upon the temperature and substratum.

(10) Perithecia - the most characteristic mature fruiting bodies of *Aspergilli* are seldom produced. Perithecia may appear accidentally in a week, or sometimes after the complete exhaustion of the medium with odd cultures. The perithecia are borne on the aerial growth. One obscure type of sporing structure is produced by these cultures, but this need not be considered at this stage.

Here it may be added that the identification of the cultures in petri plates can be readily made. The naked eye appearance of the various cultures re-

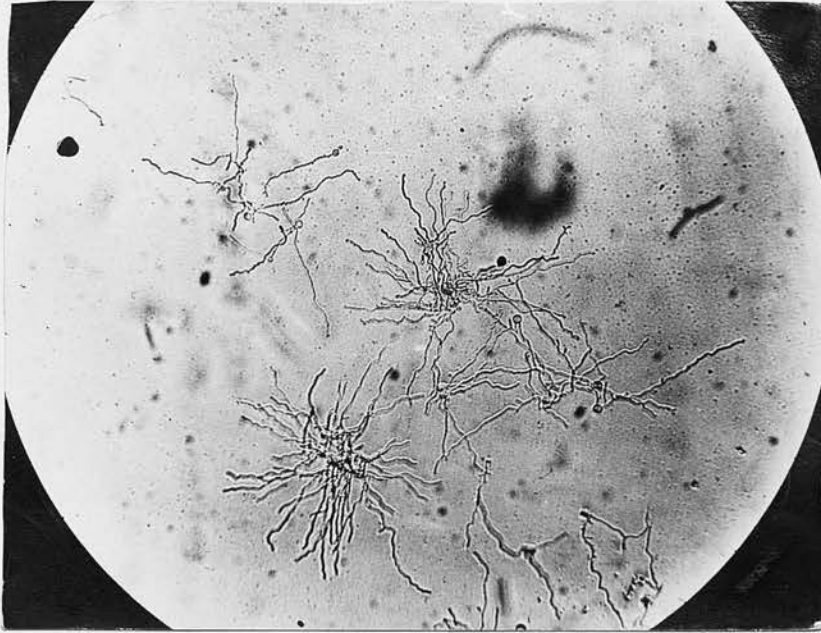


Fig.I. Culture on slide, showing the formation of the thallus from individual conidia.

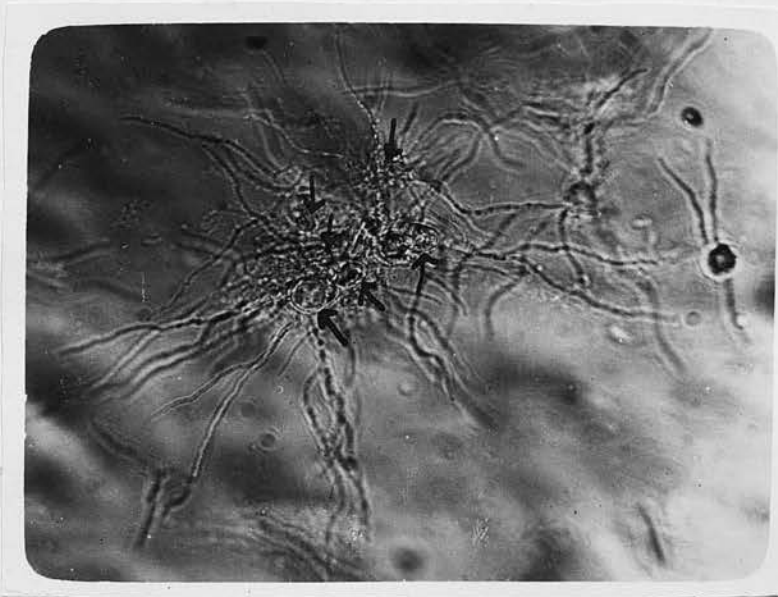


Fig.II. Culture on slide at a later stage, showing the production of large capsuled, epithelial cell-like chlamydospores. These bodies are frequently detected in diseased tissue.

covered from different sources of haematuria material has been invariable and characteristic. It is therefore possible to see at a glance whether the cultures are pure or contaminated. In case any contamination has taken place, obvious changes in the character and rate of growth of the cultures are seen. The gradation of changes through which individual cultures pass from day to day is uniform in all the cultures, the limits of variation being only restricted. The changes in the colour of cultures, and the appearance of such features as transpiration water, sclerotia and crystals, with advancing age has followed the same sequence in all the cultures.

Coming to the finer details, it is necessary to mention a few particulars about the techniques employed. Direct preparations can be made by removing young cultures on to a drop of lactic acid, glycerine, alcohol or water. As the fungal structures are not readily wetted with watery solutions a previous treatment with a drop of alcohol may be useful. A cover slip may be applied and the preparation examined microscopically. A drop of cotton blue may be added by the side of the cover slip. Again minute colonies can be scooped out together with some of the adherent solid medium with the help of a hot platinum loop, and planted on a clean slide. If the slide is then gently warmed the medium slightly melts and the culture

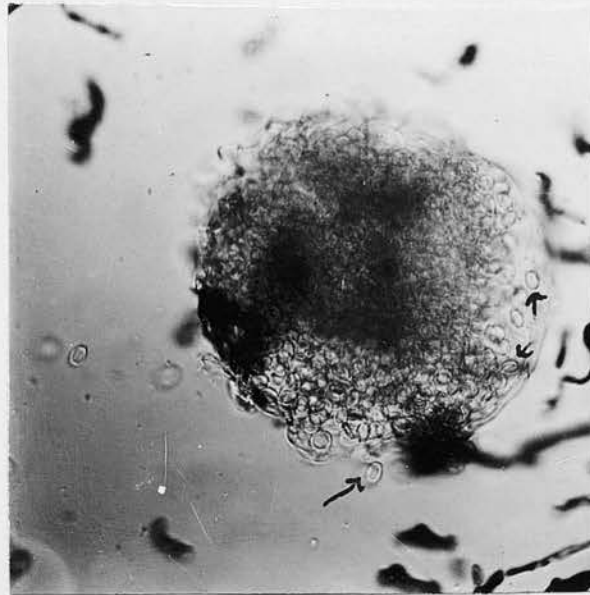


Fig.I. Perithecium from an old culture on maltose-agar of *Aspergillus* from haematuria case. The character and appearance of the contained ascospores may be noted. Perithecium 130, ascospores  $6.2 \times 4.5$  microns.

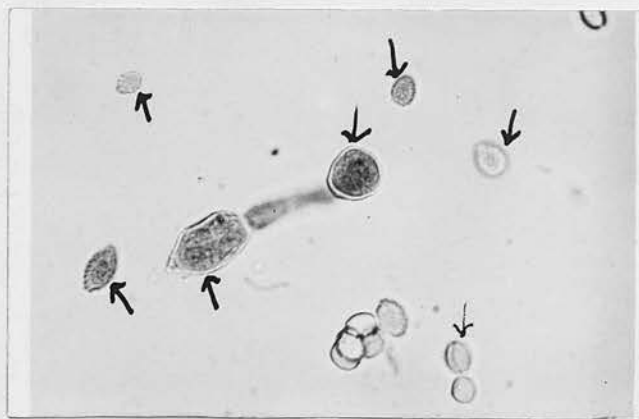


Fig.II. Smear from the same culture as above. Showing three hyphal cells attached together, of which the two terminal ones are considerably enlarged. The inner morphological details may be noted. Of the other bodies, the grooved ascospores on the right-hand side of the field may be compared with the other larger bodies with a somewhat irregular surface, the conidia. The surface of the conidia of the *Aspergillus* in question may appear smooth, or in old cultures or with special methods, considerable pitting may be brought out to view. Sometimes the enlarged hyphal cells may become triangular with three grooves at three poles simulating to an extent pollen grains. X 650



is flattened out on the slide. Cultures can also be grown directly on the slides or cover glasses, containing a very thin and uniform film of 2% agar medium. Experience shows that special provision should be made for ensuring the presence of sufficiently high amounts of moisture all the time for uninterrupted growth to take place. It is said that members of A. flavus series require about 15% moisture to start germination, the optimum being over 50%. If smears have to be made from cultures, a certain amount of displacement of fungal connections is bound to take place. One of the chief difficulties of making permanent preparations is the readiness with which conidia and even hyphae may be washed away during the processes involved in fixation and staining. It has been found that workable preparations can be obtained by drying and fixing smears made in plain water, while smears in serum or egg albumen are very unsuitable. To prevent the risk of the conidia being washed away, a protective coating of weak collodion may be used. This interferes only slightly with the staining of the fungus. It may be added that a prior treatment with a weak solution of potassium permanganate or osmic acid, or chromic acid improves the stainability of fungal structures. Fleming's fixative is the most suitable and Heidenhain's iron haematoxylin is the best stain for cytological details. Acid thionin solution may

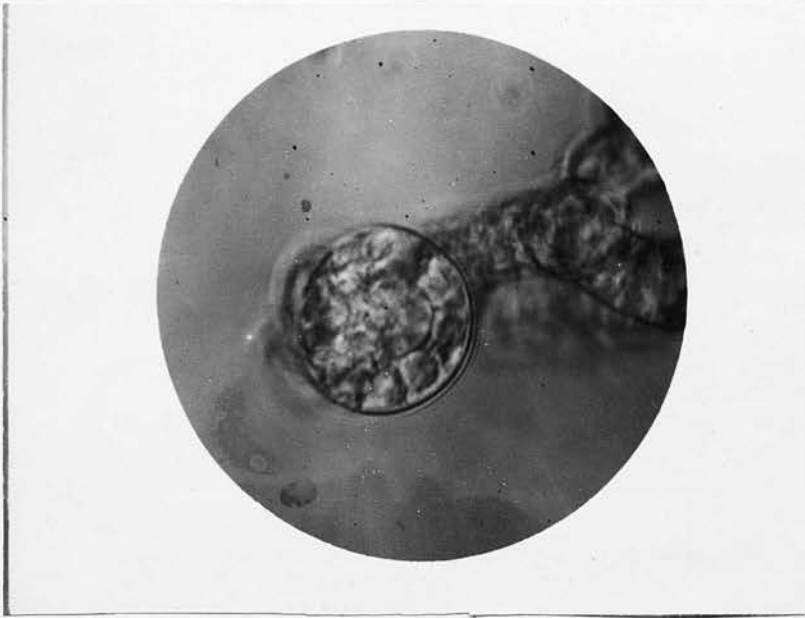


Fig.I. Terminal hyphal cell in a culture of *Aspergillus*. Note the great enlargement, rounded appearance, the peripheral row of rounded bodies arranged around a central large ring. The significance of these structures is not clear at present.

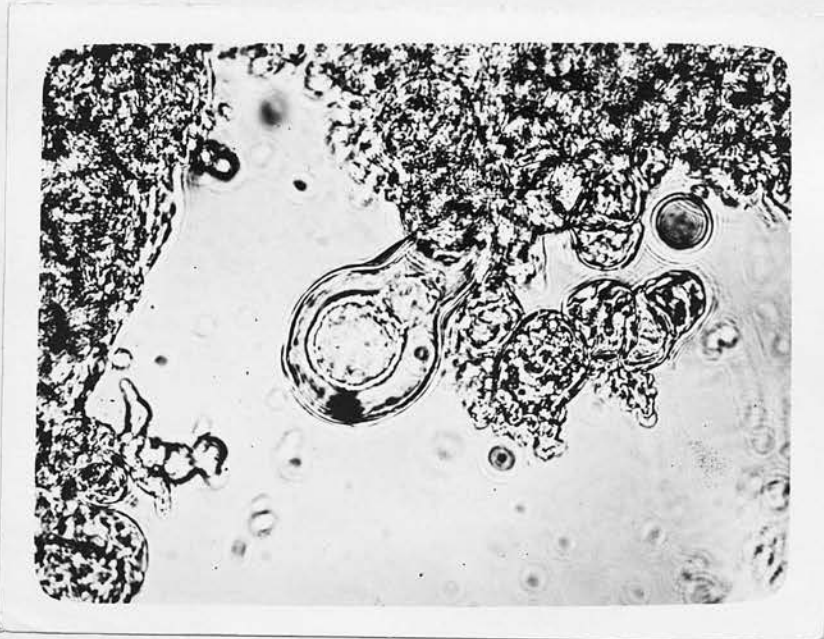


Fig.II. Same as above at a later stage. The terminal hyphal cell here is considerably more enlarged, the inner ring more prominent, the row of peripheral bodies unrecognisable. The occurrence of these peculiar stages of the fungus has to be kept in view in studying sections of diseased tissue. It will be interesting to ascertain the appearance of Hülle cells, in tissue.

also be used for staining. For mounting these preparations chloroform-balsam appears to be more satisfactory than xylol-balsam. Chromic acid produces distortion of the conidial capsules, depending upon the strength that is used.

Like other *Aspergilli*, the *haematuria* fungus shows the characteristic arrangement of its conidia and conidiophore. The conidiophore is undoubtedly unbranched, but in some aberrant forms the vesicles are particularly abbreviated and an apparent branched system may be simulated. The vesicle is generally always well-developed, and readily recognisable at the origin of the sterigmata. The majority of conidiophores present a single verticil of sterigmata. The enlarged vegetative cell on the hypha forming the characteristic foot cell may be quite prominent, but when grown in some media or at some early stages of growth the foot cell may appear to be absent. While the presence of the foot cell and the vesicle distinguishes the conidiophore of the *haematuria* parasite from that of a penicillium, the aberrant forms are such that a confusion is most likely to be caused. (Plate 64, Fig. II).

The conidiophore is septate, but when just growing the septation may not be well-developed. The stalks, under certain conditions of old cultures reveal prominent 'echinulations'. The conidial heads are mostly globose, with fairly large vesicles. The conidi-

dial chains may be uniformly and closely arranged radially around the vesicle, but the vesicle may be only dome-shaped with the conidial chains being set on the convex side. The conidial head may be composed of three to six sterigmata, a wedge-shaped vesicle, and a single conidium on each sterigmata. The colonies of cultures contain some conidial heads with markedly columnar appearance.

(Plate 56, Fig. I)  
The proportion of the globose to the columnar heads appears to vary on different media. The sterigmata may be loosely arranged in the dome-shaped vesicles. The conidial chains of columnar heads are rather closely aggregated into compact masses, and in dried cultures compact globular masses of conidia, simulating those of Gliocladium, are often found. The heads are at first unpigmented or green, and finally may become brown, or buff. The sterigmata are usually in one series, but the same head may show occasional secondary sterigmata. The conidia may appear to be smooth but the majority are roughened or 'echinulated', and evidence of superficial granulation and crystalline concretions is sometimes found.

(Plate 62, Fig. II)  
The conidiophores are occasionally very long. Regarding the secondary sterigmata, the writer feels that strictly speaking, those produced by the haematuria parasite are the original sterigmata which have become a conidiophore giving rise to two or three other conidiophores in series. The vesicles of the secondary conidiophores are generally



well differentiated. The length of these secondary stalks may vary considerably. In the denser parts of the mycelial network, the conidiophores may form half-coil like structures. This tendency to form coiled hyphae has been seen in several cultures, but though they were observed for a considerable time no perithecia were produced. In spite of the repeated attempts made by employing modifications in cultural conditions and nutritive constituents, perithecia were not produced in any of the cultures. On three occasions however, during the course of about three years they were accidentally produced under circumstances somewhat obscure. Under the influence of a bacterial contaminant a culture repeatedly produced them, though there was no doubt about the identity of the *Aspergillus* concerned. When the contaminant was separated the property of perithecia formation was again lost. In another instance, bread cultures of the *Aspergillus* were sealed<sup>ed</sup> and allowed to remain uninterfered with. When the culture was opened numerous perithecia were found, and subcultivation established that the culture was still pure. The perithecia, as already mentioned, were detected in sections of diseased bladder, and also once in haematuria urine. The walls of the perithecia are rather firm and composed of polygonal cells. The asci were oval and provided with grooves (Plate 60<sup>62</sup>). In some perithecia numerous asci were present, while in others they were few, the

majority having been already discharged. Bodies simulating perithecia to some extent but having no clear asci were also seen. These might have been aberrant and sterile perithecia or only young stages. Attempts to crush perithecia with a view to count the ascospores contained in an ascus failed. With the naked eye, the perithecia appeared, on the surface of the colony, as small dots of orange colour. It is said that pathogenic *Aspergilli* only rarely produce perithecia. When the bacterially contaminated culture was planted out on a variety of media, perithecia were produced in all. Prune extract agar produced the most luxuriant perithecia. In regard to the above morphological features, all the twelve cultures gave uniform results.

In regard to the actual measurements of the various structures forming the *Aspergillus* thallus, the following data have been obtained:-

Conidia -- size varies considerably, the smallest measured being 3.6 microns and the largest being 7 microns, the commonest varying between 5 and 6.

Conidial heads-- the smallest were about 8 and the largest about 50 microns.

Vesicles-- the smallest were 6.6 and the largest 34 micrn.

Conidiophores -- smallest were 65, largest about 500, and the commonest were between 300 and 350. The stalks from the origin gradually broadened towards the vesicle, being 7 in breadth at the bottom and about 20 microns

at the top. The sterigmata were between 7 to 10 microns in length, a few were even between 10 to 12.

Perithecia -- measured in their diameter between 72 and 135 microns. Those in sections varied between 80 and 103 microns.

Ascospores -- the smallest were about 4 microns and the largest about 16 in length, the majority appeared to be between 6 and 7.

The above measurements were taken from Czapek-Agar cultures, and it was found that considerable variation in size resulted by the use of other media. A dozen measurements were taken in each case. It may be added here that according to Thom and Church, in A.7 <sup>flavus</sup> group heads of certain species show a consistent range of measurements and form, heads of small diameter forming columnar masses, others have globose and large head. Besides in this group, several sizes and shapes of head, as stated by them, are to be found regularly in the same colony. The range of size and shape however remains characteristic.

#### Ferric Chloride Test.

This test was carried out with all the different haematuria cultures and the blood-red colouration was obtained invariably. Control tests with A.niger and A.repens from stock cultures were simultaneously carried out and negative results were obtained.

### Identification of the Cultures.

A perusal of the many descriptions on Aspergilli which have so far been recorded impresses one with two outstanding facts. Firstly the genus has not been sufficiently studied, and secondly that the naming of the various species has in many cases resulted not so much from any substantial data as from fancy or tradition. The wellknown monograph by Thom and Church has no doubt cleared the ground considerably by the broad grouping of all the described Aspergilli, but it cannot be denied that only the foundations of a satisfactory classification has been laid and that not more than a mere building has been started. The biochemical facts which have emerged in connection with some of the groups have not only vindicated the principles of Thom and Church's grouping but have contributed towards a simplified classification, which is amenable to checking by chemical tests. The fact that the colour production is a comparatively stable character in each group notwithstanding the wide range of pigmentation which are produced by different members within the group, allows of even a naked eye identification of some cultures to be made. Sufficient clear evidence exists to show that the pigments produced by the A. glaucus series are not produced by any other group. Further on this character alone, this group is divisible into three sections depending upon whether one or



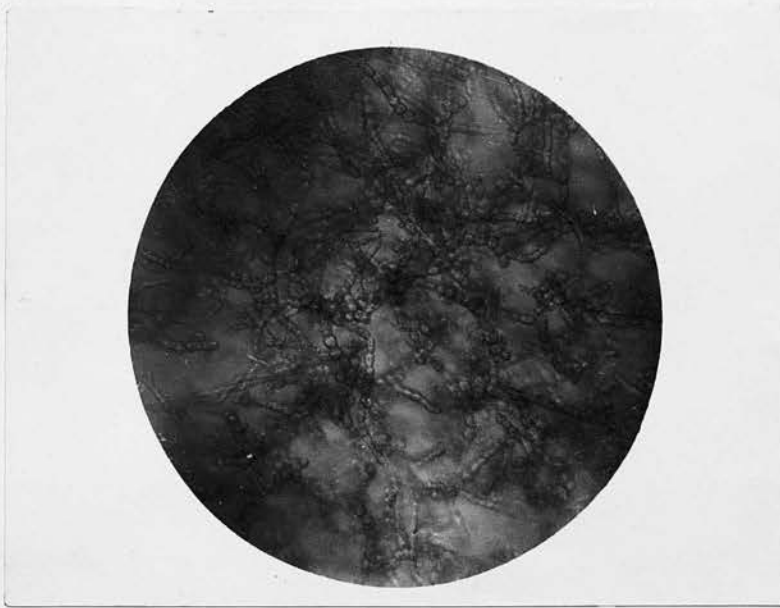


Fig.I. Old cover-slip culture of *Aspergillus*. Note how the mycelial filaments have been practically wholly converted into chains of spores. This process has also been detected in diseased tissue sections. X 198.



Fig.II. Culture of *Aspergillus* in 25% glucose solution. Shows conidial chains in an atypical conidiophore. Similar heads have been detected in bladder sections, and these may be misinterpreted as belonging to other genera of fungi (e.g. *Penicilli*) unless one is careful and knows that *Aspergilli* do produce 'conidiophore penicilloide' (Henrard 1934).

the other of the pigments, flavoglaucin, auroglaucin, and rubioglaucin is produced. Other equally interesting biochemical facts are available in support of the modern classification of this genus. Coming to other groups of this genus, there exists a simple chemical test for identifying members of the groups A. flavus-oryzae and A. tamarii, based upon the blood-red colouration which is produced by ferric chloride when added to cultures containing kojic acid, a specific metabolic product of these groups. This ferric chloride reaction indicates the constitutional resemblance of kojic acid to that of a true phenol. The character of pigment production among Aspergilli is thus of diagnostic import. The other invariable criterion for differentiating the different groups is the morphology of the specific spore-bearing apparatus, the conidiophore.

The identification and classification however are not so simple in some cases as might appear from the above remarks. A case in point is well illustrated in the series of cultures now under investigation. It will be remembered that when the same identical cultures were examined by different well-trained mycologists, a considerable difference of opinion regarding the identity of the cultures resulted (pp. 307-309). The explanation for this apparent disparity can be considered elsewhere. It is however necessary to realise the inherent difficulties and guard against un-

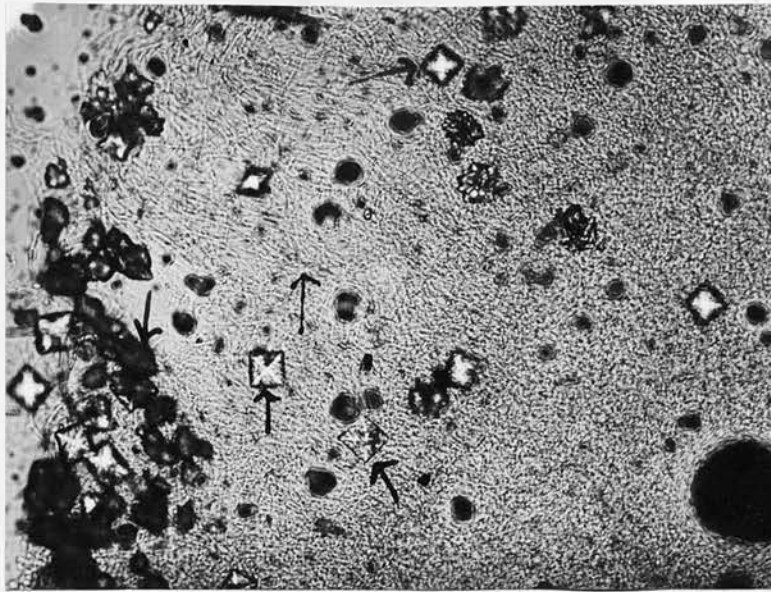


Fig.I. Crystals of calcium oxalate and earthy phosphates in the surface pellicle culture of *Aspergillus* on liquid medium (Horse digest medium). Note the extremely fine character of mycelial network forming the background. When this type of mycelia lies intertwined with the animal tissue, after the space and conditions required for the production of reproductive bodies have been exhausted, almost in a temporary symbiotic association.- the difficulty involved in the differentiation of the parasite from the host may be appreciated. X 70.

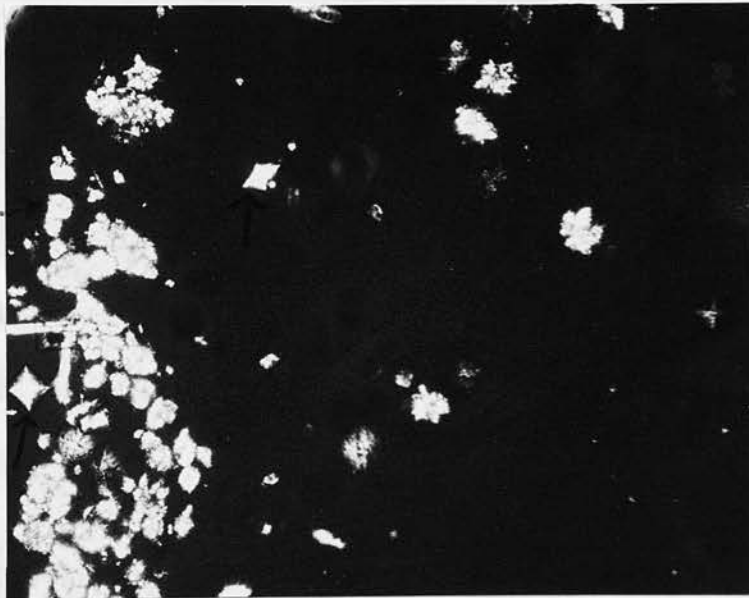
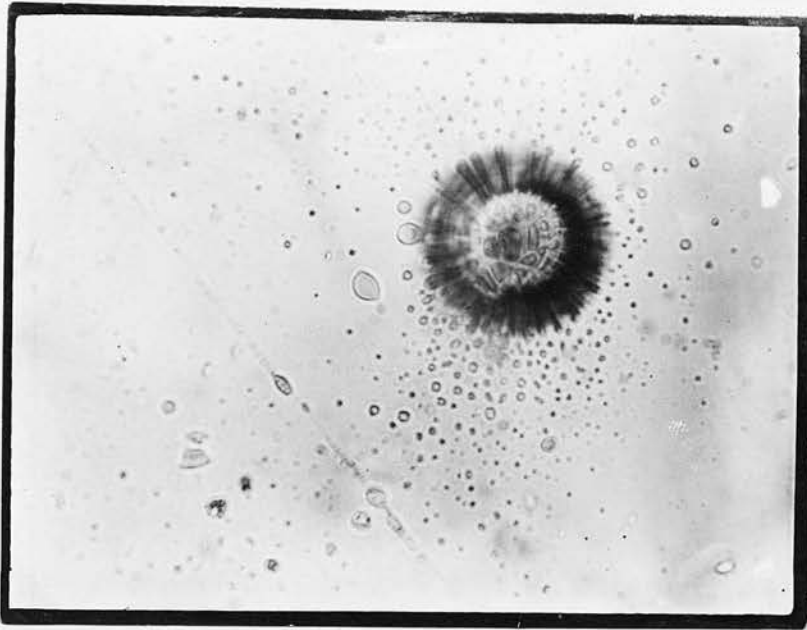


Fig.II. The same field as above, under polarised light, showing up the doubly refractile crystals of the oxalate and earthy phosphates. X 70.

due reliance being placed upon criteria of value within the groups to extend to the sphere of group separation. It must not be forgotten that *Aspergilli* are generally highly pleomorphic, and are liable to undergo variations and mutation fairly frequently. Further the invariability of the specific chromogenic and conidiophoric characters of each group is dependent to a great extent upon the use of standard medium. The failure to observe this important precaution has nullified some otherwise very interesting work. Regarding the significance of the gradation in pigmentation seen in stages of growth of *Aspergilli*, it may be remarked that the green colour of *A. flavus* series is lost on exposure to ammonia vapour but restored again with acetic acid. The intensity of green in the growing colony is a measure of the degree of acid reaction set up, and the fading of the colour to yellow and finally to brown with the advance of the growth indicates the reduction in acidity and increase in alkalinity. The actual colour of a culture thus results from a balancing of these opposing reactions. Thom and Church have rightly pointed out that the wide range of shades of mixtures of yellow and green characterising the *A. flavus-oryzae* group are to<sup>be</sup> attributed to racial limitation strain by strain in the range of hydrogen-ion concentration induced by metabolism. As already pointed out by others and recently emphasised





In tissue sections of Aspergillar infections, rosettes, or radiating clubs suggestive of actinomycosis, have been reported by Obici, Nicaud(1928), Nanta (1928)and others. In tissue sections from rabbits and cattle, inoculated with the haematuria Aspergillus, similar radiating "Actino" bodies are frequently found. The mucoid cyst in the air sinus of the skull of haematuria subjects sometimes reveal the same picture, though perhaps the bodies are slightly smaller in size than those seen in artificial lesions. The illustration above was obtained to show the simultaneous production of radial tubes from a globular body, almost instantaneously on the addition of a few drops of water to a cover-slip of a hanging drop culture which dried off. This body would seem to represent the characteristic feature of sterigmata extrusion of Aspergilli from the vesicle. In Penicillia, the sterigmata do not appear simultaneously but successively. In the course of histopathological studies, the writer has formed the opinion that the radiating bodies of Aspergillois are not identical with the clubs of actinomycosis, but are vestigial,(being without conidia), structures composed of the vesicle and the sterigmata.

X 140.

by Smith (1938), when scores and hundreds of strains are examined the sharp lines of demarcation disappear and instead of a few definite species, there is a perfectly graded series. "For most purposes", therefore, Smith adds, "it is sufficient to be able to identify any particular culture as a member of one of the group series - so-called lumping of strains".

Thom and Church (1926) have provided two short Group Keys, one based on the colour of the heads and the other abbreviated from their larger Synoptical Key. It is necessary now to apply the two Group Keys to the cultures of haematuria Aspergilli. Firstly according to the artificial Key based on colour, the following considerations lead to identification:-

- A. Conidial heads green or yellow green.....B  
 BB. Stalks pitted (often rough as seen with low magnification).....H  
 H. Stalks pitted (often appearing to be rough or asperulate with low magnifications)...A. flavus-oryzae group.

If the other Key is applied, the result is as follows:-

1. Heads not clavate.....15  
 15. Stalk walls pitted or rough, sometimes also granular.....220  
 220. Stalk walls pitted sometimes also granular, often reported as rough or echinulate when observed with low magnifications.....221

221. Colonies some shade of green or yellow green..290
290. Green or yellow-green colonies(rarely with no green colour), without colour bars but with walls marked or grooved with winding pits, often giving an echinulate appearance under low power.....292
292. Sterigmata both simple and double; mostly double in same heads; green colour usually abundant.

.....A.flavus series.

If the grouping is taken down further by the application of the Synoptical Key from 292 on page 250 of Thom and Church's Monograph, the result is as indicated below:-

292. Sterigmata partly simple but commonly in 2 series in the same heads or in different heads of the same colony.....296
296. Sterigmata often partly 1 series, partly 2 series in the same heads, especially the larger heads297
297. Sclerotia reported.....305
305. Sclerotia reported.....306

Thom and Church (1926) divide 306 into a,b,and c, thus: a. "Wilhelm adds the observation of "sclerotia minuta nigra tuberosa" to the citation of Brefeld's culture in Rabenhort, Fung. eur., Edit. Nov., Ser.II. 2135.....A.flavus.

b. Colonies fairly green (Kronberg's green on sugar media); stalks usually 400 to 700 microns long, rarely 1,000; conidia pyriform to globose varying from

2 by 3 microns to 5 by 6 or longer.....108 & 3526

c. De Bary and Woronin transferred the species to Eurotium.....E. aspergillus flavus."

The haematuria Aspergillus would appear to conform to b. and c. more closely than to a., since the sclerotia examined were not 'nigra'. While the conidial size agrees with that of b. the stalks are somewhat smaller. The colony colour is no doubt Kronberg's green. The only member of the A.flavus series which is definitely known to have produced perithecia is c.. De Bary and Woronin called the organism Eurotium aspergillus flavus, and Thom and Church (1926) appear to have accepted the validity of the species. From the description available in the original publication, which is rather vague, it is not possible to make any detailed comparison. Having studied only twelve cultures, the writer feels that for the present a more minute classification is not indicated. As emphasised by Smith (1938), it is sufficient to know that the cultures obtained from different haematuria sources belong to the A.flavus series.

On pages 307-309 of this treatise, the preliminary reports on the writer's cultures as obtained from different mycologists have been quoted. It will be noticed that the first two cultures, obtained from two different animals were considered to be identical and coming close to A.tamarii. The same officer later added that



the cultures were to be placed in A.glaucus group, between A.ruber and A.scheelei. The next culture was considered by another mycologist to belong to the Ver-sicolor group. When two further cultures were examined by a different officer, the identification was that the two cultures belonged to the flavus series of A. flavus-oryzae group. Later still Mr. George Smith at London considered that eleven of the writer's cultures belonged to A.flavus Link. The exact considerations upon which the different mycologists came to apparently dissimilar conclusions are not known, but one may try to explain how this could have happened. Firstly, the ferric chloride test applies to the flavus-oryzae and tamarii groups, secondly the brown pigmentation and sclerotia formation may be common to both. As Thom and Church have pointed out, aside from colour, A.tamarii resembles A.flavus in size of colony, habit and appearance. As a differential character, the absence of green colour in the former may be mentioned. Turning to the resemblance of A.glaucus to A.flavus, it will be remembered that Mangin (1909) suggested that the separation of these groups was difficult. Thom and Church have however placed them at two ends of their group separation. The common bonds between them are the green colour of their conidia, the pitting or echinulation of the conidia and the conidial sizes. Regarding the differentiation, soft-walled quickly ripening perithecia characterise the glaucus to the exclus-

ion of sclerotia, while reverse is the case with the flavus. Regarding the common bonds of the versicolor and the flavus, the conidial heads are green or yellow-green in both, and some of the morphological features are common. The character of the stalk however differentiates one from the other. Further it is necessary to remember that mutations and variations are a frequent occurrences among Aspergilli. Thom and Church state that the A.flavus group is capable of great variation under more or less definable culture conditions. After maintaining a culture of A. effusus Tiraboschi they were able to obtain one of the common types of A. flavus, and this finding has been confirmed by Burnside. Regarding the classification of individual strains, Funke(1929) makes a very interesting statement, which is typical of others and may be quoted here in extenso: "But we must bear in mind that the genus Aspergillus and especially the group flavus-oryzae present great difficulties on this point. I fully agree with Boedijn, when he writes: "While it is not difficult to arrange them under the big groups of this genus classification of the frequently numerous by-forms is practically impossible. Even with the new monograph of Thom and Church, these by-forms cannot satisfactorily be defined "and also" I have relinquished the idea of giving separate names to the races, (though B. admits that: Even slight differences in the races proved to be con-

stant in culture"). It is doubtful whether such a thing would be any good in the case of *Aspergillus* with its numberless forms for every large species. Yet it is not to be denied that it might have been justifiable for a few types in the *Aspergillus flavus-oryzae*".

"There is no reason why any strain, isolated from any spot on earth and from any source of food, should not probably prove to be constantly different from the strains already known. It becomes a hopeless enterprise to give them names. For the present, we have to consider the *flavus-oryzae* group as a collection of hereditary races, the number of which cannot be estimated at this moment. As sexual propagation does not occur in *Aspergillus flavus-oryzae*, we can only imagine that they have come into existence by mutation, as Schiemann suggests". The terms, *Euaspergillus flavus* and *Sterigmocystis flavus* have also been previously used for this group.

In view of the statements of Smith(1938) and of Funke(1929), the dominant and invariable associate of haematuria need be called *A. flavus* series, though its relation to *Eurotium aspergillus flavus*, De Bary and Woronin is close. It must be added here that the haematuria cultures have behaved uniformly in biological tests, including optimum temperature of growth and pathogenicity in rabbits.

VIII. ASPERGILLOSIS.(a) Previous Literature and a General Resume.

Animal experimentation with Aspergilli may be stated to have started with Grohe in 1870, who planned his experiments to disprove the statement of Villemin that tuberculosis was a specific inoculable disease. Emulsions of fungus spores were administered into animals in various ways. Firstly, rabbits were injected intravenously with 2/3 c.c. of an emulsion of fungus "spores", assumed to be spores of Asp. glaucus, Penicillium glaucum, and yeasts. Tubercle-like lesions were produced in internal organs and mycelial threads were detected therein. Similar tubercles were set up in the white and grey matter of the brain, in the choroid plexus, in the vitreous humour, the retina and the choroid of the eye by the introduction of spores into the carotid artery of the dog and the lamb. Intraperitoneal inoculations produced lesions in the diaphragm and internal organs, including the liver and the kidneys. Similar results were obtained with intratracheal injections, and in one case the caseous lung lesions failed to show fungal mycelia. Injections into the knee joint were also made, and though a simple



inflammatory process was no doubt set up, any mycelial development could not be detected. Similarly, inoculations into the subcutis and into the anterior chamber of the eye were carried out, but the results were not quite clear. In the same year the above experiments were again carried out by Block, who generally confirmed the results.

The subject was restudied by Grawitz in 1877. It was surprising that none of his two hundred dogs and rabbits died as a result of infective inoculations, and he failed to observe any marked interference with the well-being of his experimental subjects. He was therefore led to conclude that his experiments had completely failed to confirm the previous work. Later, when he destroyed an animal injected three to four days earlier, he found localised parenchymatous nephritis of the kidneys, while other organs showed nothing unusual. The degree of degeneration varied with the vital activity of the fungus strain used. He found that while a patch in the first stage of degeneration showed no indication of its fungal origin, a patch of more advanced degeneration revealed small hyphal tubes under caustic potash treatment. He ascertained that the fungal hyphae are disintegrated and disposed of in about eight to fourteen days of the inoculation and no indications of their

origin are left behind. Contrary to his earlier results, he succeeded in producing fatal results in rabbits in seventy-five hours, and in dogs in a hundred hours, after the administration into the blood stream of fungi which had been grown repeatedly over a length of time on an albuminous, alkaline, liquid medium, maintained at 37°C. Typical lesions, characterised by mycelia and cells in a state of degeneration, were produced, and in no case were the mycelial filaments seen to proceed to the stage of "spore" formation. He formed the opinion that he had succeeded by his method in transforming a non-pathogenic fungus to a pathogenic variety, and also that he could raise the virulence of his organism with each subculture on the special medium, the microscopic characters of the tissue changes in experimental animals depending upon the degree of exaltation of the virulence of the strain used. This opinion regarding the possibility of inducing an initially harmless strain to assume pathogenic behaviour, was changed as further experience accumulated. Autopsical examination revealed that in an affected animal, certain organs, like the kidneys and the liver, exhibit the aspergillar lesions more frequently than other organs, like the brain and the lungs, and he offered an explanation of this selective action based on the

relative oxygenating power or physiological activity of the spores to that of tissue cells in the various organs. Further, Grawitz attempted to immunise animals experimentally by the use of killed fungus strains, and with minimum doses of virulent spores. The chief difficulty encountered would appear to be the standardisation of the initial immunising dose, for while a weak dose did not raise sufficient response from the tissues, a strong dose caused death of the treated animal. In a number of animals, however, he believed to have succeeded in producing something like an absolute immunity, for, when these were subjected to inoculations with enormous quantities of virulent spores after initial "immunisation", not a single mycotic lesion was set up.

The subject became so interesting and promising that a number of workers were attracted by the problems. Koch and Gaffky, Loeffler and others challenged Grawitz's conclusions. These subsequent workers showed that Grawitz had started with a fundamentally wrong impression that all fungi were naturally non-pathogenic. They pointed out that his cultures were made without precautions against contamination, and further that they probably contained a few "spores" of a pathogenic *Aspergillus* which increased in numbers with each sub-culture, explaining his exaltation

experiments. They showed that the spores of what they then wrongly termed Aspergillus glaucus (later found by Eidam to be A. flavescens), and spores of another Aspergillus, which was unidentified by them but later proved by Lichtheim to be A. fumigatus, were virulent without any preparation, irrespective of whether the cultures were grown at the room temperature or in the hot incubator. Koch emphasised that Grawitz's experiments had failed to prove any successful immunisation, since in the autopsical examination made after the second injection or test dose, numerous lesions containing young mycelia were discoverable, and indeed these lesions were extensive enough to be seen with the naked eye. Death was caused not by an increase in the interstitial nephritis left behind from the initial immunising dose, for besides other reasons, Lichtheim obtained identical results, even after allowing a full month to elapse between the two injections for the recovery from the lesions of the first injection to take place.

The disparity between the results of the contending parties (Grawitz and his opponents) was explained by Lichtheim by discovering that there are two green Aspergilli to be found growing on bread which differ in their biological and physical characters. By



repeated examinations, he found that the one which grew much more quickly at the room temperature, and not in the hot incubator, and which proved to be avirulent on injection into animals, was the common variety generally present on vegetable substances exposed to the air. The primary virulent strain was, however, much rarer. The former was identified by De Bary as A. glaucus (Eurotium glaucus), and the latter organism as A. fumigatus, corresponding exactly in character with that found by Fresenius in the air passages of the bustard. De Bary had already encountered A. fumigatus growing frequently in the hot incubator, and rarely at ordinary temperatures.

Lichtheim observed that when the green culture, being a mixture of both the species, was maintained at the high incubator temperature, it caused the pathogenic species to overgrow the saprophytic, and when brought down to ordinary room temperature it was still pathogenic. He added that the culture could become non-virulent by an inverse substitution, by growing at the ordinary temperature.

Eidam described later the characters of A. (Sterigmocystis) nidulans, which is very pathogenic to rabbits, and Laulanie, while investigating the vascular origin of true tubercles, showed that the lesions of the lungs of rabbits, produced by the

injection of spores of A. fumigatus, corresponded very closely to those of true tuberculosis affecting the same animal. Fraenkel made attempts to attenuate the virulence of this organism by maintaining it for six months at the high temperature (51°-52°C.) at which the production of "spores" is inhibited, but without success. Olsen and Gade found that different species -- A. subfuscus and A. fumigatus -- possessed different degrees of virulence for the rabbit. Lindt studied the effect of injecting the ascospores of A. nidulans into animals, but obtained only negative results.

Ribbert, 1886-1888, investigated the "disappearance of pathogenic mould fungi in the animal organism." He observed that if the dose of A. flavescens inoculated into an animal is not sufficient to kill it, the leucocytic reaction which is stimulated exerts a potent influence on the character of the lesions produced by a second injection. Further, that the leucocytic response was greater, sharply limited, and the development of the fungal elements was less and restricted, but no actual immunity was produced. Proceeding to study the effect of repeated injections, he confirmed the above findings. He noticed that if cultures are not subcultivated for several months and allowed to dry, their virulence to experimental

animals and growth energy are both reduced, and this feature is continued over several generations. These observations with regard to A. flavascens were confirmed by Hugemeyer, and in respect of A. nidulans and A. malignus, by Lindt. Ziegenhorn maintained his cultures at various temperatures and showed that no marked attenuation of virulence resulted from this procedure, provided the temperatures were not such as to kill the fungus altogether. In 1890 Heider made an attempt to determine if the injection of Ascospores would produce any difference from that produced by conidial emulsions, but only identical symptoms and lesions were produced. The liver and the lung showed nothing abnormal to the naked eye, but large numbers of ascospores were found to have commenced to germinate in the microscopic lesions.

In the same year, Dieulafoy, Chantemesse and Widal showed that the aspergillar lesions in pigeons were similar to those of tuberculosis, excepting for the presence of mycelium instead of the tubercle bacilli. A natural case showed the presence of an infected grain forming the centre of a tubercle in a bronchus. In the anterior chamber of the eye, infected experimentally with A. niger, Delephine found a white and opaque material, different from pus, and composed of epithelial-looking cells and a few swollen spores. Intraperitoneally, numerous tubercles were produced in

the viscera including the back and sides of the bladder, but no general peritonitis resulted. The tissue in the liver nodule was much altered, and the cells had completely degenerated. In none of the lesions was any typical mycelium seen and only slight tendency to germination of spores was present. Notwithstanding the active changes that were present no other organism were found in any lesions. Each collection of spores was surrounded by a zone of more or less altered small round cells which were no doubt leucocytic, and these were enclosed by a layer of young and adult connective tissue. Three years later, Ernst stated that it was comparatively common to find A.fumigatus growing in the bronchi of diabetic patients, and Kotliar in the same year failed to establish the existence of toxins in A.fumigatus cultures.

In a thesis dated 1894 and a subsequent series of papers, Renon recorded the results of his comprehensive investigations. He recorded the passage of the A.fumigatus in the urine after twenty-four to forty-eight hours of experimental inoculation. He cultivated the organism on Raulin's fluid and demonstrated it in stained centrifuged deposits. At autopsy, fairly frequent lesions in the bladder and typical renal lesions were detected, but the ureters appeared to be unaffected. The lesions were ascribed to infection by the venous system. In attempting to grow cultures on



urine, he found that the spores and mycelium had little tendency to vegetate under those conditions.

Renon found that curiously dogs and cats were particularly resistant to the aspergillus, which was so highly pathogenic to pigeons. In infected grains the spores were found only on the surface and not in the deeper portions. The spores of *fumigatus* were found in the air, on leaves and on barks of trees. No certain results were obtained in his attempts to immunise animals. By administering spores by the mouth as well as intravenously, lesions in the small intestines and the caecum were produced, those by the former method being intramucous while those by the latter were submucous. With the intravenous method, the lesions were larger and composed of a caseous central mass with a peripheral layer of delicate mycelium surrounded by embryonic cells. No giant cells or any ulceration were seen. In the rabbit fed with the spores, lung lesions were present, together with a perforated and ulcerated intestine and some ulcerations higher up in the gut. With large doses of *A. niger* spores being given to rabbits no lesions were detected at autopsy, but the animals became obviously emaciated. In his comparative studies, the leucocytes in the *fumigatus* lesions commenced to take up the spores only feebly at about the third hour and the classical tubercle was not produced until after the

twelfth or the eighteenth hour, while the spores of the niger were found to be completely enclosed at the end of three hours. The leucocytic reaction being so rapid and intense for the niger, and so reduced and slow in the fumigatus, explained the pathogenicity of one against the comparative harmlessness of the other.

Similarly Obici studied the effects of fumigatus spores in pigeons and found that while numerous greyish tubercles resulted in the liver and the lungs, the kidneys and spleen were only congested while other organs appeared to be normal. He found numerous small collections of leucocytes in the liver and active desquamation of the endothelium of blood vessels. A pigeon fed with the spores showed no gross lesions, but the intestinal submucosa was definitely infected. In rabbits the subcutaneous route was found to be unfavourable but rare deaths occurred due to diffusion of the aspergillus in consequence of plentiful injections. A localised reaction of small cell infiltration around unchanged spores but no mycelial activity was seen. In other experiments the nerve cells changed into shapeless heaps of protoplasm and granules. Filtrates of the culture were tested for toxin, but no local or general reaction was evinced. In experimental lesions the mycelia were always sterile, and never were any fruiting hyphae pre-<sup>sent</sup>-/excepting in the lungs where 'actino' rosettes and stars were seen.

Degenerative and necro-biotic changes in internal organs were found. Following intravenous injection, the liver was more often affected in birds, <sup>while</sup> the kidney suffered most in mammals. On feeding spores, they were found to pass through the lymphatic vessels of the intestine.

In studying the typical aspergillar tubercles, Rothwell observed that the protoplasmic contents of the mycelial thread had a granular appearance, and ascribed it to some necro-biotic changes. He found individual spores had given rise to a small sprout, and 'Sporangia were also present', though most of them were manifestly degenerating. In lesions in some situations the mononuclear and polymorphonuclear leucocytes were present but the presence of the fungus or in any case the presence of the spores was not revealed. The examination of a tubercle in the liver capsule showed it to be composed of spindle-shaped cells, the resemblance to a sarcoma being very close indeed. In guineapig lesions he found that the muscles in many places had undergone hyaline degeneration while in others they were completely degenerated. The spleen formed the seat of multiple haemorrhages, and in places the tissue was literally torn through. When A.niger spores were placed in skin pockets, several were found to have germinated giving rise to slight projections and in one to a long sprout. The

majority of Rothwell's experiments were by the intra-peritoneal route. This was chosen to minimise the mechanical effects of embolism, which might follow an intravenous administration. Although previous observers failed to prove any toxin production by A.fumigatus, one is bound to admit, he added, that both the fumigatus and the niger form toxins within the body. While both, experimentally were capable of producing similar histological reaction, injection of the fumigatus killed animals but the niger never did. The fumigatus was more capable of germination in the liver tissue than the niger. Both organisms are very resistant whether living in the animal body or outside, but the fumigatus is the more pathogenic, though both can live and produce lesions in living tissues. He concluded that both A.niger and A.fumigatus in their natural state are capable of living in the animal body both as parasites and saprophytes and that A.fumigatus was the commoner of the two in either capacity. In 1905 Constantin & Lucet studied the effect of some pathogenic Aspergilli upon animals. In 1916 Greco, working in the Argentines published an account of the origin of tumours and mycoses with histological descriptions. Between 1920 - 1923 Sartory published fascicles on Fungous parasites of man and animals, in the latter year in collaboration with Bailly, he published a separate book on pulmonary mycosis and their



parasites. In 1929 Hiroshi & Morita compiled a rather comprehensive list of references published on *Aspergillus* dating from 1729 - 1928. In the same year Matsumota studied a series of *Aspergillus* cultures by serological methods, and appeared to obtain some success. In 1924 Falck & co-worker found that in the genus *Aspergillus*, black, brown, and light brown types produce acid freely, the yellow and greenish yellow produce acid moderately, while the pale and white only slightly. With protracted cultivation on a calcium containing medium the intensity of acid formation was increased. The ultimate product was oxalic acid, preceded by gluconic and citric acids. In 1929 Emmel described a natural infection of the kidney with *A. fumigatus*, in the same year Tscherniak detected *A. nidulans* in secondary rhinomycosis in horses suffering from petechial fever. Gibson in 1930 discussed cases of splenic mycosis in man, and attempted to prove that a definite aspergillar disease entity existed. In 1932 Dessy carried out some experiments on a rabbit infected with *A. fumigatus* and showed that crystal violet, brilliant green and chloride of copper possess a high degree of chemotherapeutic power. In 1932 Savage & Isa studied an outbreak of *fumigatus* infection among brooder chicks. Serious mortality took place and they traced the source of infection to the use of corn silage as litter. In 1933 Nicolaus studied the histogenesis of *fumigatus* lesions in fowls and

from the appearance of the lesions attempted to estimate their comparative age. The histological development was differentiated into three stages, subepithelial up to twelve hours, fructification phase up to three days, and period of capsule formation from three days onwards. Conidial formation took place entirely in the necrotic areas, ceased, and resumed again on the surface of nodular lesions. Characteristic siderotic encrustations (Gandy-Gamma nodules) in the spleen of dogs following inoculations with aspergilli were described in 1933 by another worker. Recently Bresciani has reported that A.niger from human patients increased in virulence with each successive passage through white rats, eventually causing death of these animals from general aspergillosis and sterigmocystosis. Nanta has recorded the production of splenomegaly in rabbits following inoculations with A.nidulans. Thom and Church (1926) have reported that in a woman A.versicolor had grown and fruited in a tightly bound breast under irritation. They add "Grain molded with strains of A.flavus has been charged with the poisoning of cattle and hogs in several instances, but experiments in which bacteria were excluded have thus far proved non-toxic." Again, "Members of the group have been occasionally found in pathological lesions and when tested on experimental animals certain species were reported to be pathogenic, but natural infection by such organisms is certainly rare in

mammals. Henrici (1930) states: "Many strains isolated from the air or vegetable matter show no pathogenicity." He adds: "Strains freshly isolated from spontaneous infections may exhibit a surprising degree of virulence. No lesions are apparent in such acute infections. With smaller doses or less virulent strains multiple miliary abscesses occur in various viscera, especially the lungs. Intravenous inoculation into rabbits usually causes death in three to five days. Multiple minute abscesses in the cortex of the kidneys are the most striking lesions in these rabbits. Subcutaneous or intraperitoneal inoculations product localised lesions which may not be fatal". "If one dusts spores into a tumbler and holds it over a pigeon's head for a minute or two, there developes a rapidly haemorrhagic pneumonia".

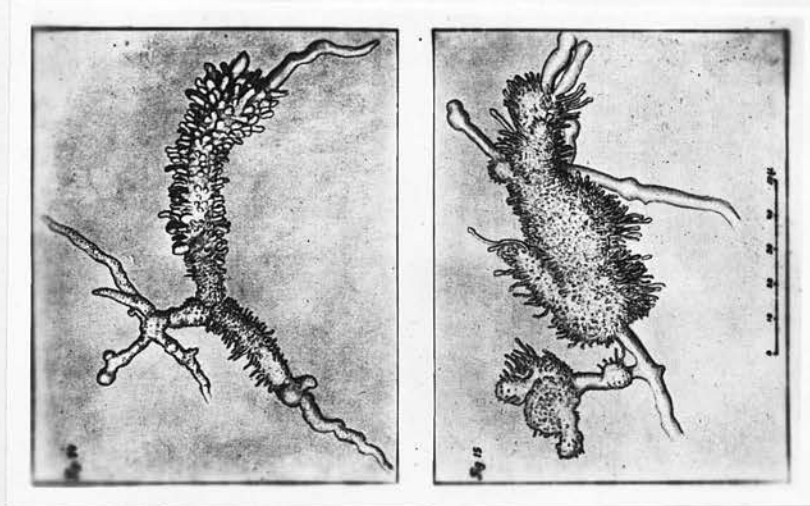


Fig.I. Reproduced from Bendixen and Plum (1929). Investigating cases of bovine abortion histologically, these workers found the above forms of *Aspergillus* in naturally and artificially infected animal tissue. These spiked, club-shaped enlargements, giving a roughened appearance to hyphal tubes, have been termed 'Kolben' in German, and are not found among saprophytic fungi or in fungal cultures, according to these authors. In the present studies on haematuria tissues, these structures have been repeatedly seen. The work of the above Danish authorities on Aspergillosis of the reproductive system is somewhat in line with the present investigation.



VIII.(b) TRANSMISSION EXPERIMENTS.

In order to confirm the successful transmissions with urinary sediments already obtained in five Hill Bulls and in a Calf (p.272,273,274), by the use of pure cultures of the haematuria *Aspergillus* (*A.flavus* series) the following experiment was carried out. Adult cattle, calves and rabbits were used, besides a few guineapigs and white mice. Death was caused in varying periods and depended upon the number of spores in the culture emulsion used. Rabbits appeared to be the most susceptible subjects. The result of the transmission experiment with pure cultures was generally in agreement with that obtained with urinary sediments.

Rabbits.

The intravenous method of inoculation was commonly used but occasionally subcutaneous injections were given with a view to study the developmental stages of the parasite in the tissue. Intraperitoneal injections and inoculations into the testicle were also carried out. The most outstanding lesions were produced in the kidneys, the liver, lungs, with an intense congestion of other visceral organs. The urinary bladder showed invariably more or less dilatation of the blood vessels, congestion and occasionally even degrees of haemorrhage in the mucosa and submucosa. On rare occasions the urine gave a positive reaction for blood by the benzidine

test. On one or two occasions at the very commencement of these studies, fairly large doses were administered and death was caused in twenty-four to forty-eight hours, and autopsy showed a very intense congestion of all the viscera with haemorrhagic urine. The usual pseudotubercles were not well differentiated. In cases where extremely small doses were given, the rabbits became ill for about eight to ten days and then appeared to be free from disease, as far as the maintenance of normal habits and external appearances were concerned. When any of these apparently healthy rabbits were destroyed and autopsied degrees of visceral involvement were unmistakable. Microscopically the presence of the fungus in the tissues could be proved. Experience gained with these rabbits showed that the longer the rabbits lived after the infection, the greater and more marked were the lesions in the urinary bladder. Definite histological lesions and fungal activity in the bladder, <sup>were detected</sup> as in to those seen in natural bovine disease, / As far as other organs were concerned the fungus was detected in the typical pseudotubercles in the lung and the liver, in the intestinal submucosa and in the spleen as well. With the naked eye the spleen appeared to be intensely congested but no clear nodular lesions were recognised. Histologically the presence of the fungus in that organ was proved. The enlargement of the organ was not very marked. Plate 71 shows the microscopic features of the

typical Aspergillar pseudotubercle and an intense parasitic activity associated with the host's reaction. The rabbit tests were repeated at Edinburgh, with a view to ascertain whether any attenuation of the virulence of the cultures had resulted, because of the cultures not being subcultivated for about a year and having been maintained in tubes well plugged and paraffined. Plates 73, 74 and 75 illustrate the lesions as exhibited by some of these rabbits, which died between seven to ten days of the experimental inoculations. The lesions exhibited were typical, and wholly agreed with the descriptions of previous workers, notably Rothwell's (1901). The lesions in the lungs revealed the radiating club-like structures, previously reported by Obici, Nicaud (1928), <sup>and</sup> Martins (1929) in aspergillosis. Though these structures resemble the specific clubs of actinomycosis, present studies have shown that they are of a different nature, being in all probability embryonic aspergillar heads, consisting of the well-developed vesicle and radially arranged sterigmata. (Pl. 66 & 69).

Guineapigs and White Mice.

The lesions produced in the guineapigs were very similar in appearance to those of the rabbit but they were perhaps more minute. Millet seed sized greyish lesions were present in the kidneys and liver. The spleen appeared to be enlarged. The fungus was isolated from the heart blood and kidney.

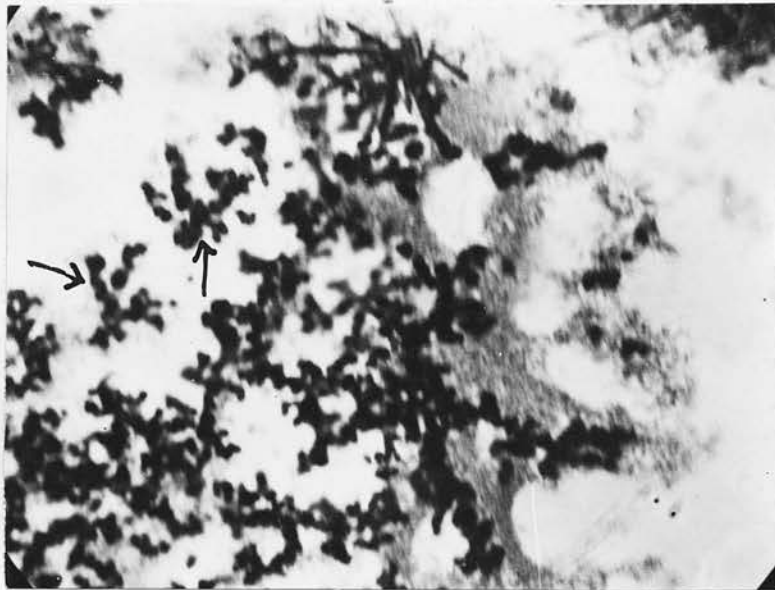
Plate No. 68

Fig.I. Section of lung. From an experimental animal succumbing to intravenous inoculation of a pure culture of the fungus. The dark stained, chained organisms are identical with those seen in natural cases, as well as in sections of potato-kidney culture. Compare with Plate No. 57 Fig. II .. X 460.

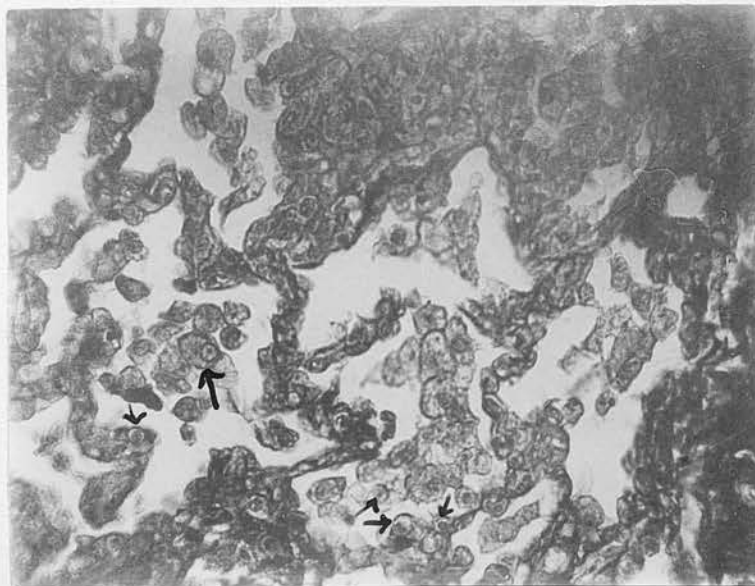


Fig.II. Section of lung. From an experimental rabbit as above. Various stages of the parasite were detected including large and small spores, mycelia, uninucleated stage with ringed nucleus and central dot, and 'actino' forms. Numerous parasites were phagocytosed. X 260.



Plate No. 69

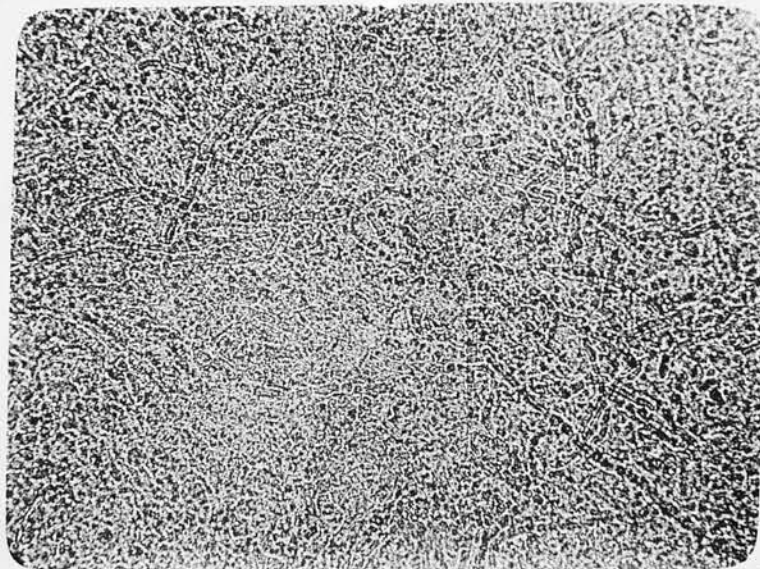


Fig.I. Tissue smear. From kidney of experimental rabbit, killed with intravenous administration of pure culture. Smear prepared by scraping, and treated with a drop of picric acid. Shows branching, segmented, hyphae.

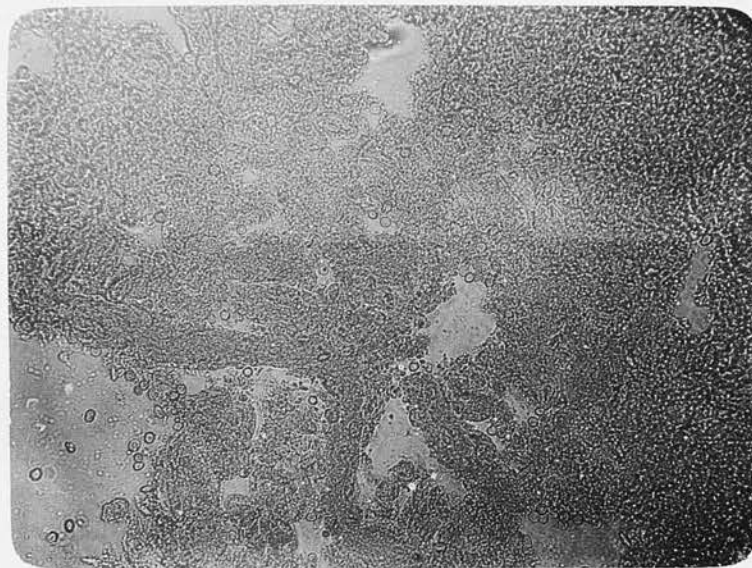


Fig.II. Another field of the same smear showing numerous capsuled conidia.

Calves.

Three calves were employed and they were given thin emulsions of the pure Aspergillus flavus series previously isolated from a clinical case. One of the animals was given subcutaneous injections at four day intervals and the other two were given the administrations intravenously. The former animal died, while the latter animals were alive till discontinued from the experiment. The subcutaneous lesions were intensely haemorrhagic and had extended deep down into the tissues. That the parasite had broken the subcutaneous resistences and found its way into the general circulation was proved by recovering the organism in culture from the heart blood and visceral organs. The lesions in the kidney and the liver were very marked. Lesions were also found in the urinary bladder, consisting of epithelial proliferation and haemorrhagic patches in the submucosa and depth of the organ. In the two animals treated intravenously, no obvious haematuria was produced but the increase in the cellular content of the urine was so pronounced that parasitic activity in the urinary system could not be doubted. One calf was given injections at fortnightly intervals and the other received only two injections but at an interval of twenty-two days. The observation was not continued for a sufficiently long period, judging by the available information regarding the incubation period of the nat-

## Plate No. 70

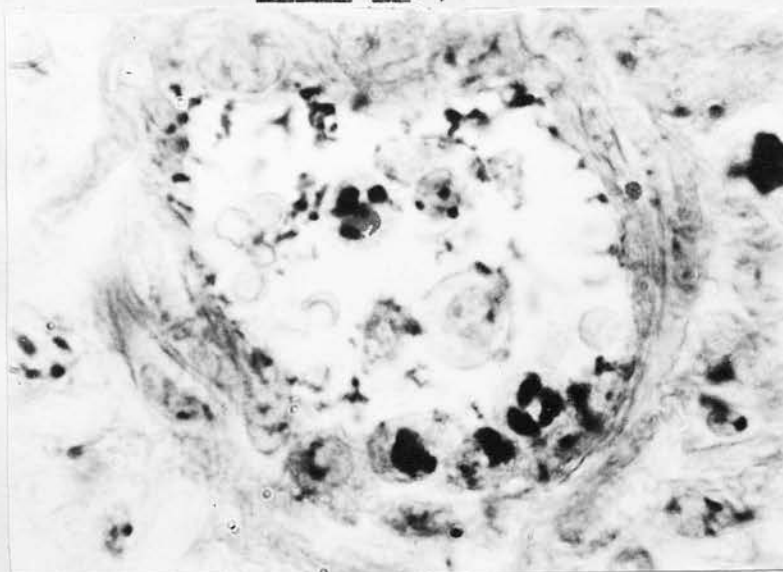


Fig.I. Section of the lung. From a bull, dying one month after intravenous inoculation of Aspergillus flavus series in pure culture. Stained Heidenhain, showing stages of the parasite in activity in a bronchiole. Note the large parasitic bodies, and half moon-shaped 'cryptococcal' shells. cf. the 'halb-mundformige' bodies described by Scharer(1930) in haematuria cases. The mycelium is not clearly shown. Similar pictures have been presented by experimental lesions in rabbits as well. X 1100.

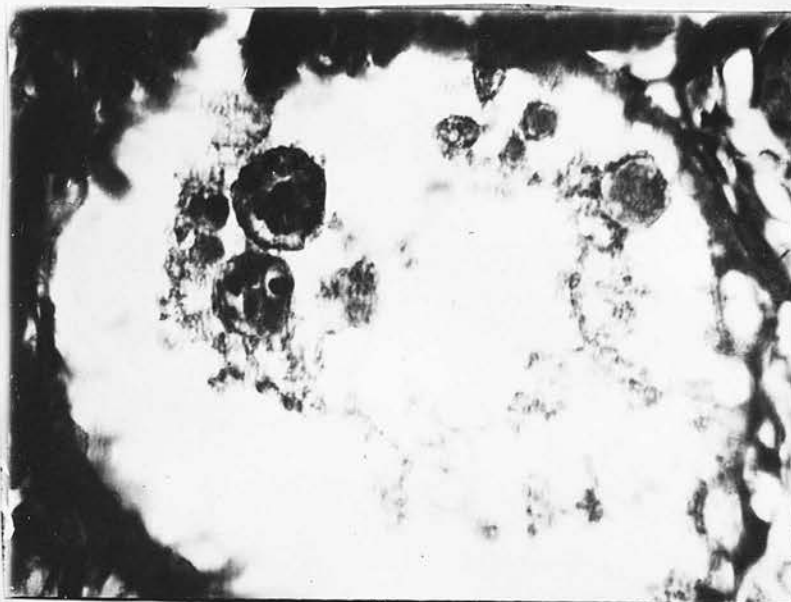


Fig.II. Section of the lung, as above, showing the Aspergillus thallus in an air cell, with strings of branching granular hyphae, bearing large globular parasitic bodies. It is not easy to say which stage of the culture tube is represented by these 'cysts' of parasitic life. Note the resemblance to the similar structures detected in the natural disease. (Plate 36, fig.I) X 350.

ural disease. It is also possible that the interval at which the inoculations were made was too long. Experience indicates that an interval of about seven to eight days is the best to adopt in such transmission experiments.

#### Hill Bull.

A healthy bull was taken and kept under observation and its urine and blood examined. This animal was given a heavy intravenous administration of a pure *Aspergillus* culture from a clinical case. After about a month of the administration the animal was extremely ill and moribund. The temperature continued to be subnormal and there was sufficient indication that the animal would definitely die in the next day or two. As already mentioned, a continued subnormal temperature for a few days before death has been an invariable feature in natural cases of haematuria. The organism was recovered in pure culture from the internal organs of this animal and the presence of the organism in intimate association with the lesions in all the organs was proved by histological methods. The internal organs of this animal are illustrated in Plates 43 & 44. The lesions are sufficiently marked to be recognisable even in the photographs.

#### VIII.(c) Histological Studies.

The most outstanding lesion in all the internal organs of these cases was the presence of degenerative



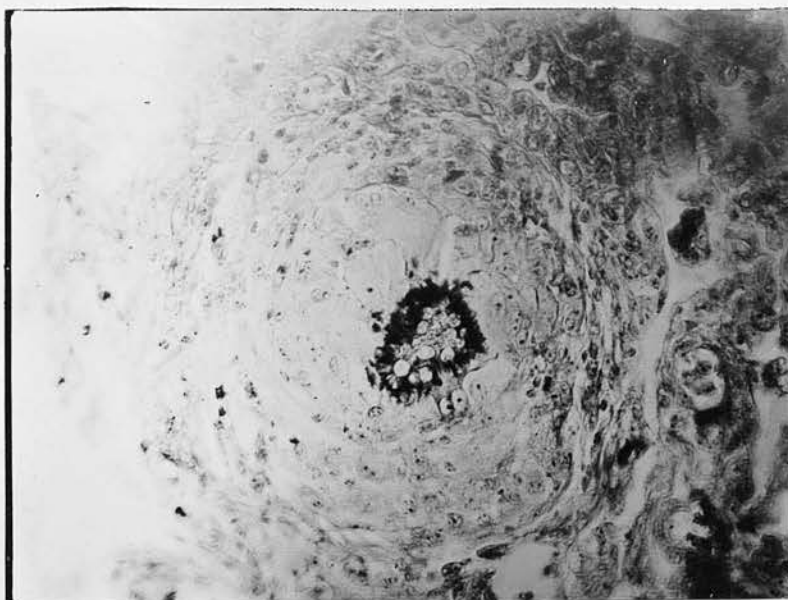


Fig.I. 'Aspergillar pseudotubercle', experimentally produced by intravenous administration of the culture. Centre contains a cluster of capsuled structures - mostly conidia perhaps. In sections, a few of these capsuled bodies show typical radiating clubs, reminiscent of Actinomycosis, around them. Present studies suggest them to be sterigmata growing around vesicles. The lesions are similar whether they be in the lung or the liver.

X 300.

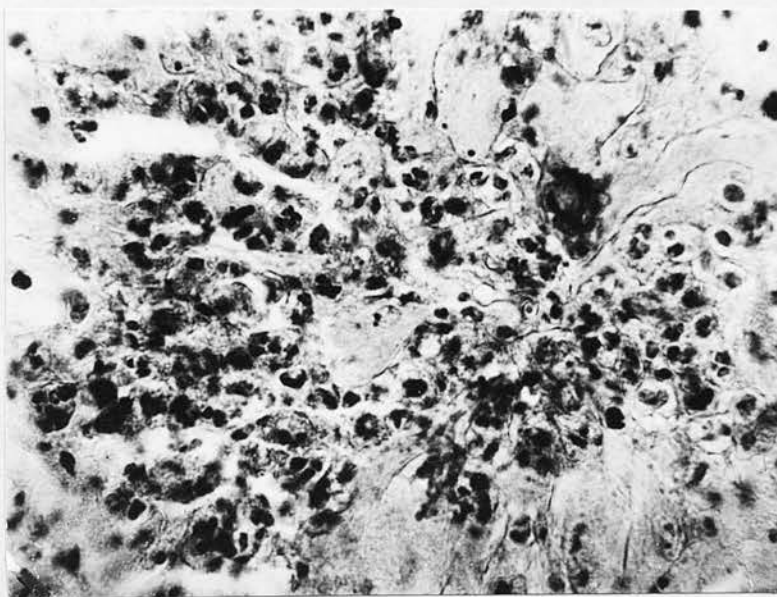
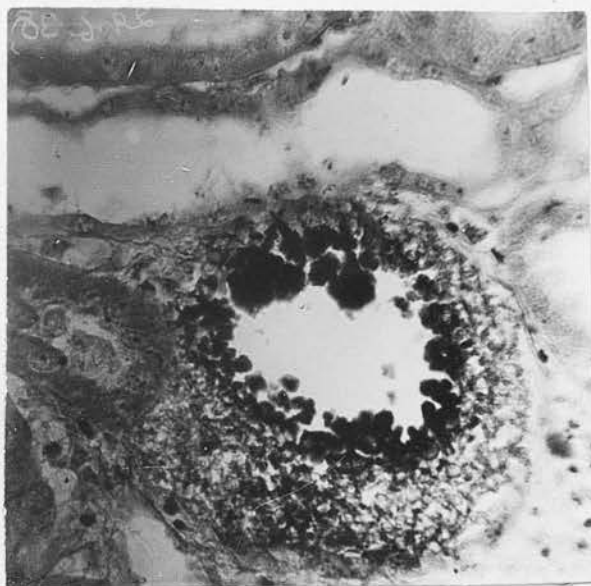


Fig.II. Section of lung, (experimental rabbit), showing the host's reaction in acute disease against the parasitic inoculum. While both the parasitic elements and the host's reaction are well represented in this field of actual fray, the difficulty of differentiating them may well be appreciated. Stained Heidenhain. X 580.

and necrobiotic changes. These were so widespread that a suggestion of postmortem changes could be made, had it not been for the fact that these changes were also present in tissues that were collected warm and fresh. Most of the organs showed intense congestion and marked engorgement of the blood vessels. Multiple haemorrhages were also rather general. Numerous collections of leucocytes around fungal spores and mycelial filaments, surrounded with forming and formed connective tissue strands were seen. In organs like the lung, liver and the kidney typical Aspergillar pseudotubercles were detected. In some blood vessels there were large masses of the fungal elements. The endothelium of the vessels was actively desquamated and considerable mycelial activity in the vessel wall evident. In some places actual haemorrhages from the vessel wall were seen to have spread out into the perivascular regions. The most outstanding picture in the blood vessels was however presented by conidial heads projecting into the lumen of vessels, the endothelium and subendothelial region forming the base of the stalk (Plate 72). The capability of the fungus to fructify inside blood vessels of living animals would appear to be an important factor enabling the organism to live and multiply at the expense of the host. It is very doubtful if this property could be possessed by any saprophytic fungi. In any case the same finding in the natural



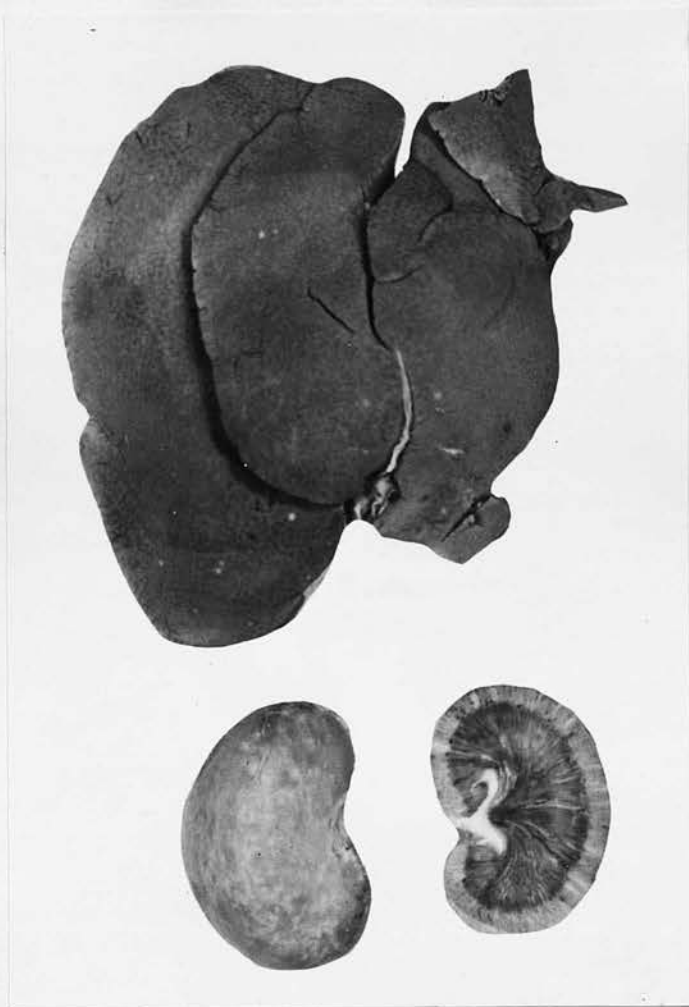
Section of the kidney. Stained H.E.. From an experimental bull, inoculated with a massive dose of pure *Aspergillus* culture, into the jugular vein. The animal died after five weeks of the inoculation and presented characteristic lesions in the urinary organs, the lung, liver, with minor lesions in the intestine and lymphatic glands. 50c.c. of the fungus emulsion was used. Naked eye lesions were prominent in the kidney, epididymis, the bladder and the liver. Histologically the most impressive picture was the presence of characteristic aspergillar heads projecting into the lumen of blood vessels with the progressive bursting of the vessel wall as a result of mycelial activity. Essentially the lesion was the same as illustrated from natural cases in Plate No.35 and Plate No.40. The picture presented here is much more pronounced than that of the similar vascular lesions of natural cases.

disease as illustrated in Plates 35 & 40 is most suggestive. It may be noted that in spite of the fact that tissues from cases of natural and experimental Aspergilloses have been studied many times, excepting the mention of atheromatous lesion by Jacobsen vascular lesions have not received much attention. The microscopic appearance of the experimental lesions in the lungs from the bull has been illustrated in Plate 70. The developmental stages of the parasite and the different morphological features can be seen. The typical conidial heads fructifying inside the blood vessels have been found in the kidneys, liver and the bladder. No actual perithecia were detected but the character of the focal lesion was the same as already described in the natural disease.(Plate 27). The intestinal lesions produced in the bull was identically the same as in the natural disease(compare Plates 43,42). The fact that in this animal mucoid cyst in the air sinus, similar to the finding in the natural disease, was found and showed the same histology is of unusual interest. Mucoid cysts in bovines have never been reported so far, as far as the writer has been able to ascertain. (Plate 4,Pl.43,Fig.9).





Internal organs of an experimental rabbit. Inoculated intravenously with 0.25c.c. of a very thin emulsion of an old culture, initially recovered from the heart blood of a haematuria bull. The culture was tubed and paraffined one year earlier, and was not opened and subcultivated for that time. This experimental test was carried out at Edinburgh to see if the results obtained in India could be repeated, and also whether the vitality and virulence of the strain were still intact. The 'miliary' pseudotubercles in the kidney and lung can be seen. The liver was intensely congested and enlarged but the nodular lesions were only few. Histologically, the typical Aspergillar lesions were found, with localised and general haemorrhages in the organs together with foci of cell infiltration associated with fungal spores, limited budding and disintegrating mycelia. The character of the lesions depends upon the concentration of the spores in the emulsion and also upon the dose administered. This rabbit died seven days after inoculation.



Internal organs of an experimental rabbit. Inoculated intravenously with 0.25c.c. of a very thin emulsion of an old culture, initially recovered from the bladder tissue of a natural case of the disease, haematuria. One of the easiest ways of recovering *Aspergillus* cultures from diseased tissue was found to be to put pieces of the tissue, collected with aseptic precautions, into 25% sterile glycerine. The growth may be slow but this method avoids any risk of contaminative bacteria interfering or over-running. Potato slants and Czapek's agar may be equally well employed, and if desired Acriflavin (one in 25,000) may be added. This however is not essential. In this particular rabbit quite a number of pseudotubercles appeared on the surface, and in the parenchyma of the liver, while the lesions in the lung were larger in size than those of the rabbit illustrated in the preceding page. This rabbit died nine days after the inoculation. The kidneys showed that the individual localised pseudotubercles were not so well circumscribed, as the previous case, but the organs were more tense and showed a greater extension of the disease. Lung not shown above.



Internal organs of an experimental rabbit, dying after ten days of receiving intravenously 0.25c.c. of a thin emulsion of *Aspergillus* culture, recovered from the heart blood of a calf. This calf was only a few months old, healthy and in good condition. It was used in experimental transmission work. Subcutaneous injections of a pure strain of *Aspergillus*, 5c.c. doses, were administered in front of the axillary region commencing from 26.12.37. The injections were given at 4 day intervals and repeated for about 17 times, when it died on 24.3.38. The lesions exhibited by the rabbit treated to this calf culture were less circumscribed and more generalised, particularly as far as the kidneys were concerned. This rabbit died 10 days after the inoculation, the fungus therefore had a greater and a longer opportunity for its invasive and lethal activity than in the two fore-going rabbits. The pseudotubercles appeared to be larger than in the other two cases. Similar lesions in the lung, liver and particularly the kidney were encountered in guinea pigs and rabbits inoculated with urinary sediments from haematuria cases, but the lesions were not so acute and generalised.

IX. FUNGI AS ETIOLOGICAL AGENTS OF ANIMAL DISEASE.  
Discussion.

This discussion can be commenced with advantage with a brief outline of some peculiar problems outstanding in this field.

Mycology, the scientific study of fungi, has hitherto been outside the purview of the medical or veterinary biologist. Consequently clinico-pathological work upon mycotic diseases has suffered considerably from a lack of mycological precision. The developmental forms of fungi encountered inside the animal body have not been investigated by systematists, but it is remarkable that their knowledge, as gained solely from studies upon artificial laboratory cultures, should have been transferred unquestioningly into the domain of animal pathology, and assumed to apply wholesale to the biology of fungi in animal tissues. Obviously if the results of in vitro studies are to apply to in vivo conditions, it must first be established that completely identical environments exist in the two substrata, particularly as fungi are known to respond even to the slightest differences therein. It is true that in their metabolic activities fungi synthesise organic compounds from inorganic sources, and break down organic substances, but there is as yet no proof that these biochemical activities in the outside world are identical in degree or even in quality to those in



tissues. To complicate matters, there is besides the question of the reaction in animal tissues to contend with, depending upon the nature of the host's reaction to a pathogenic fungus in its healthy tissue, or to a mere secondary invader in an already diseased tissue. With alterations in the cultural conditions, in oxygen tension, or the duration of infection, variations are likely to take place; and it is known that even among bacteria such as the anthrax bacillus the size for one thing may fluctuate considerably. Fungi can withstand unfavourable conditions, and organisms which are usually strict aerobes in the outside world can live and even multiply under diminished oxygen tension in living or diseased tissue. These facts must signify that adaptations are brought about in the fungal constitution, both in morphological forms and physiological tendencies, to make life possible under adverse conditions. Fungi are capable of certain types of enzymic activity and fermentations, and this fact applies to the Aspergilli of the flavus group in particular. The knowledge on enzymes of A.flavus-oryzae is still fragmentary but protease and amylase have been found. Birkinshaw (1931) showed that in this group alcohol is regularly produced but not in the A.glaucus group. The utilisation of urea and tannin by Aspergilli and similar other reactions have received prolonged studies. The disease producing properties of a fungus

must no doubt be in conformity with its life history. It appears that the mechanism of fungal activity in animal tissues could be defined in precise chemical and physical language, but so far studies appear to have been restricted to test tube investigations.

In order to appreciate the extent of our ignorance on many points which are more or less fundamental to the understanding and control of disease problems, it is necessary to take a few concrete instances. To start with the causal relationship of a number of fungi to disease entities has been definitely established, but it is remarkable that the actual source of infection in practically all cases is still unknown. The fungi are ubiquitous, the majority are saprophytes growing on either soil, plants or other organic matter, and being non-pathogenic to man or animals. Besides there are fungi which are comparatively harmless invaders of the natural openings of the animal body, and others again which grow in external or internal seats of disease. There are quite a number again, which are definitely pathogenic to man or animals, while some attack even insects. In pathogenicity tests, certain fungi prove highly virulent, (seldom isolated from the air or vegetation), while it may be extremely difficult to effect successful transmissions under artificial conditions with species known to be definitely pathogenic under natural conditions. Some animals subject-

ed to the same risks of infection apparently remain unaffected, while others readily contract it. The pre-existing set of circumstances constituting a predisposition to fungal infections in individuals is still unknown. In universal experience mycotic infections can be surprisingly refractory to treatment and progressive over many years, but it is said that under the conditions existing in the living body reproductive spores are not usually produced and vegetative stages are not particularly vigorous. Cases are known of the persistence of dermatomycosis after vigorous treatment and yet the available methods of examination failed to reveal any evidence of the infection. The detection of fungi when present as only single cells in the tissues without mycelial development has always been difficult and their interpretation as protozoa the commonest mistake (*Coccidioides granuloma*, blastomycosis, sporotrichosis, *Histoplasma capsulatum*). Some of these infections are still included in well-known text-books on Protozoology. These can be cultivated and their mycelial stage studied, but the reason of the absence of that stage in the tissue remains unexplained. In addition to these 'dimorphic' fungi, there exists another type (*Rhinosporidium*), where the infective agent still remains to be cultivated and its mycelial stage revealed (presumptively 'monomorphic'). The situation is much more complicated among the *Aspergilli*, since the

genus is characterised in its single cell and multi-cellular stages by a diversity of forms including conidia, vesicle, Hulle cells, sterigmata, sclerotia, perithecia and ascospores. There is no indication in literature as to whether one or the other of these stages is modified or altogether skipped under the special conditions obtaining in parasitic life. A considerable amount of controversy has raged regarding the single cell stage of fungi, including those of *Aspergilli*. It is certain that yeasts are not produced by *Aspergilli* and that *saccharomyces* are not converted into mould fungi (Schioenning & Kloecker, Seiter, Wehmer, Wortmann, Pouchet, Hellier, Juhler), but as Jacobsen (1932) has pointed out in his text book, "In the tissues the *Aspergilli* lose their characteristics and may be observed only as mycelial threads or roundish or oval yeast-like bodies, the typical fructifications are absent in the tissues". "Exceptionally, as in *P.camemberti* and some *Acaulium* sp., the hyphae may break up into oidia or again in *Acaulium* sp. form gemmae or, as in *A.niger* or *A.oryzae* (Schramm, 1914, Zikes, 1922) develop sprout mycelia". Henrici (1930) admits that Oida and yeast-like cells are not peculiar to any class of fungi, being formed under certain circumstances by members of *Phycomycetes*, *Ascomycetes* and *Basidiomycetes*, and he adds that yeast-like cells are <sup>not</sup> specific characters. It will be appreciated therefore that these trans-



itional forms (vegetative oidia, conidia, actively vegetative yeast-like cells, dormant chlamydospores) as detected in infected tissues may be the source of much confusion. From the practical standpoint, therefore, mycological studies as specially applying to pathology must include primarily features of the organism exhibited during growth in actual animal tissues under conditions approximating, as closely as possible, those existing in the animal body. The state of information available on other points such as the production of toxins by various fungi, the mechanism of pathogenic action, immunity responses and therapeutics is equally unsatisfactory. It is remarkable that while on the one hand organisms closely allied in mycological classification may produce widely different animal responses, the same or nearly identical lesions may result from organisms at two extreme ends of such grouping, on the other. So far the tenets of classification have failed to take cognisance of such fundamentally different facts of biological life, as are represented by wide divergences of tissue reaction. To remedy the above unsatisfactory state of knowledge on fungi in their pathological implications, the subject of what is now known as Medical Mycology has developed comparatively recently, thanks to the efforts of Castallani, Henrici, Redaeli & Ciferri, and Dodge in this direction. It is to be hoped that the need for adopting appropriate

techniques in myco-pathological work will come to be realised, since at present sufficient attention does not appear to have been devoted to special methods being required for bringing out the fungal thallus in optical sections. The Gram-negative fungi and the differentiation of the vegetative from the reproductive parts of the thallus in tissues do not appear to have been investigated to any extent.

In his text book on Pathology, MacCallum (1936), describes the above unsatisfactory state of affairs in this field thus:

"The great confusion which exists in the literature on fungus disease is largely due to the enormous field - Saccardo has recorded some 57,000 species - but more specially to the fact that many synonyms have been invented for each form and, still worse, that the same name has been given to quite different organisms. It is quite impossible for any one who has not spent years in the special study of these parasites, to speak with any assurance".

The actual disease entities which owe their origin to fungi may be divided into external and internal mycosis, consisting of dermatomycosis, otomycosis, respiratory infections and mycetoma. General infections due to fungi are also occasionally met with. Some of the well-known infections are ringworms, actinomycosis, blastomycosis, torulosis, aspergillosis, moniliasis, sporotrichosis, rhinosporidiosis, and epizootic lymph-

angitis. Some of these diseases are transmitted by direct contact, and others through the mouth or the upper respiratory tract. The pathological effects of fungi include abscesses and ulcers, punctate haemorrhages, areas of degeneration or necrosis, verrucose or papillomatous proliferation of the epithelium, myxoma-like cysts, pseudotubercles and granulomatous changes. A form of hypersensitivity to mould allergens may also be set up. Growths simulating cancer have also been encountered in mycotic infections. Fungi may affect the epidermal and subdermal structures. The lesions in the internal organs may be localised or spread to other parts through the blood stream or the lymphatics. The exact manner in which the tissues are damaged by fungi is not clearly known. The occurrence of tubercle-like lesions associated with giant cells suggests that the organisms behave as mechanical or foreign agents. Acute lesions simulate those of streptococci and indicate that some toxic action may be in operation. Several attempts have been made at different times to ascertain if toxins are produced by pathogenic fungi. No general agreement has yet been obtained. Experimenting with yeasts, Neumayer found that if no fermentable substances were introduced at the same time no disease resulted, otherwise a gastro-enteritis could be set up.

Among the aspergilloses, a tendency to recurrent

and marked haemorrhages in an otherwise well-nourished subject, long duration and comparatively stationary symptoms are noteworthy. Of the sixty or so species of aspergilli reported as affecting the skin and viscera of animals, A.fumigatus appears to be the most serious. Infections with this parasite take place among poultry, penguins, domestic animals and even in man. Among chicks, epidemics of brooder pneumonia can be very fatal. Eggs and through them, the embryos can be infected during incubation. Among adult birds, the disease is contracted through eating mouldy grains and due to damp housing conditions. A superficial epithelial infection of the air sac, diffuse infiltrative lesions, or nodular pseudo-tubercles may be produced in the lung. Primary disease of the lung due to aspergilli is known to occur among animals and man. Infections of the skin (pinta, tokelau), nails, the ear, lung, intestines, and spleen have frequently been described. The lung infections appear to be common in Germany and France among persons exposed to large numbers of conidia (in pigeon feeders and hair sorters). It is said that in the ear, aspergilli utilise the ear-wax, and that the fatty covering of eggs predisposes to the infection. Some authorities believe that primary aspergillosis of man is rare, the majority of reported cases being thought to be secondary to other diseases, of which the evidence has been obliterated by the asper-



gilli. Lesions in the conjunctiva, in the air sinuses, and ulcerated granuloma of the hand have been described. An orbital neoplasm resembling a sarcoma has been proved by Wright (1927) to be actually an aspergillar granuloma. In veterinary practice, mouldy forage and silage have often been suspected to be the cause of animal disease, but the results of experimental work has been uncertain. Church and Buckley (1923) concluded that though pure cultures of the common saprophytic moulds are not generally toxic when ingested by laboratory animals, mouldy food was objectionable for both man and animals. They added however that by feeding pure cultures of moulds common in food products the suspicion of toxicity in food spoilage being connected with moulds could be eliminated. The flora of silage has been studied, and most of the moulds present were found to be the common species, known to be harmless. Eckles, Fitch and Seal (1924) state that Aspergilli are the only species in silage which could cause sickness in animals but practical experience exonerated them.

Some of the mycotic diseases can be highly destructive and extensively generalised, e.g. human blastomycosis, actinomycosis. Regarding the latter, one authority states thus: "Burrowing through the tissues for great distances, completely distorting whatever it traverses and it stops for nothing - bones are penetrated as easily as muscles - and from the lung such a mine-like advance may push through the pericardium and heart wall

into the interior of the heart." In a number of the more chronic mycotic diseases, the organisms may be unstainable and show up merely as shells, while in the fresher and less resisted infection rapidly budding forms may be detected in the scattered visceral lesions of the liver and the kidney. The lesions are composed of mononuclear and epithelioid cells in association with the organisms. In chronic fungal infections the mononuclear reaction is common while in acute cases polymorphonuclear activity is the more prominent. The parasites in the lesions sometimes comprise of only very minute, round or oval bodies and no evidence of mycelia may be detected. In some infections 'the spores may not greatly differ from blood corpuscles in size or in appearance'. Some of the spores may appear as ill-defined vacuolated bodies. The mycelia may be filamented, rosary-like or disarticulated. From their experiments, Blakeslee & Gortner(1913) suggest that "there may be a possible relationship between *Rhizopus* and some of those diseases such as pellagra, the corn-stalk disease, and the horse disease of the Middle West, the causes of which are at present unknown, but which have been supposed to be due to infected food". In studying the reaction of rabbits to intravenous injections of mould spores(*M.hiemalis*), they gave in 1915 an enormous number of spores but autopsy failed to reveal abnormal conditions of the viscera, or accumulations of spores.

ON  
TREATMENT.

Present position.

The treatment of the disease has been attempted at many places. The available evidence indicates that encouraging results may be obtained in prophylactic measures and in the curative treatment of early and mild cases only. The disease, once established, is incurable. The same criteria have not been used for the interpretation of the results of treatment carried out by individual workers. It is important to differentiate between a definite cure and the passing of the animal to the quiescent stage of the disease. According to Moussu, well established lesions cannot be cured, though occasional cures may be possible. Hadwen states that cure is not the rule, and that in all the experimental animals kept under his constant observation, numbering sixty-six, not a single case lived over five years after showing the initial symptoms.

Various methods of treatment have been tried by different workers. Successes have been claimed with haemostatics and vaso-constrictors, it being supposed that the cessation of the passage of blood indicated true cures, whereas in such a naturally intermittent disease, permanent and complete cures should have been aimed at. Workers who have had the experience

of treating a number of cases in various ways, make the recommendation that the only sound economic measure to adopt in this disease is to fatten the affected animal for the butcher. It has been observed repeatedly that in spite of the passage of quantities of blood and the persistence of the disease, animals can be fattened readily in most cases. A useful precaution, not noticed by others, has been emphasised by Hadwen, to the effect that the affected animal should be tied up and kept as quiet as possible. A variety of drugs have been used, including lime salts, iron and tonics, Neosalvarsan, trypan blue, Naganol, Urotropin, Adrenalin, calcium lactate, tartar emetic and many others. Among recent workers, Durin and Unglas (1931) treated several cases in France with repeated doses, orally and hypodermically, of a B. coli vaccine. On the analogy of the treatment of human genito-urinary infection with B. coli, colloidal silver was used by Unglas (1932). Like his predecessors, this worker failed to follow up his observations and to check them finally by a careful autopsy and histopathological examination.

More recently, Schlegel (1934) treated twenty-eight cows and five oxen with vigantol, chemosan, clauden, stryphnon, vitakalk, etc. The action of stryphnon was found to be comparable with that of



adrenalin in this disease, but more lasting results were recorded. Merck's ephidralin in 9 c.c. doses produced similar results. Of the above treated cases, fifteen cows and three oxen are said to have been cured, while ten cows and one ox had to be slaughtered. In order to compensate for the loss of blood in the urine, a quantity of blood from a healthy cow was transfused into a clinical case, but no amelioration resulted. Butozan (1938) employed oral therapy with alum, leaves of uva ursi, iron preparations, and irrigated the bladder with alum solutions. Further, he observed amelioration independently of medication. The provision of fodder sufficiently rich in calcium and vitamins with the addition of molasses has been recommended by Schlegel. The provision of dietary supplements to produce a favourable Ca:P ratio has been tried, but some of the treated cases remained incurable. According to the experience of most workers, the removal of animals to haematuria-free localities does not produce any relief in protracted cases, while a few workers, notably Lienaux (1919), Lockett (1932), and Bankier (1936) affirm that cures can be effected thus in early cases.

Attempted Treatment of Clinical Haematuria Cases.

The following clinical haematuria cases were under observation and treatment with various drugs,—Azamine, Stovarsol, Carbarson, Bin. Iodide of Mercury, Tincture of Iodine, Adrenalin, Formalin, etc. The drugs were administered either subcutaneously, intravenously or orally, and either daily or at varying periods. Of the drugs tried, the intravenous administration of Formalin appeared to be encouraging in some early cases, while in others it seemed to aggravate the advanced lesions, precipitating a crisis and death. Adrenalin has often been found to be very useful as a haemostatic and stimulant, and as postponing the serious symptoms of collapse and immediate death.

(1) H.B. 74 (clinical case), aged 8 years. Died on 15-6-36. This was a very debilitated animal and a chronic sufferer of haematuria. It was treated with a variety of drugs, including Azamine, Stovarsol, Iodine, Carbarson, Bin. Iodide of Mercury, Adrenalin and Formalin, as follows:--

Commencing from 12-9-34, at 4 or 5 days' intervals, given intravenous injections of Azamine in doses of 100 c.c. of 0.5% Sol. of Azamine for about 2 months, the injection repeated 14 times, the last injection being given on 15-11-34. Four pills of

Stovarsol in about 100 c.c. of water given on 22-5-35, and repeated on 23rd, 24th, 25th and 26th of March, 1935. 6 pills of Carbarson administered on 26-4-35, and repeated on 27th, 28th, 29th and 30th of April, and 1st and 2nd of May, 1935. Bin. Iodide of Mercury with Pot. Iodide administered thrice daily from 1-2-36 to 3-36. Gradual increase of blood/<sup>loss</sup>noticed during the course of above treatment. Subcutaneous injection of 3 c.c. of 1:1000 Adrenalin solution on 26-2-36. Repeated on 28-2-36. Blood in the urine was markedly decreased. Intravenous injection of Formalin in 120 c.c. of N.S.S. on 26-3-36, repeated on 30-3-36, 3-4-36, 8-4-36 and 15-4-36.

Urine. Haematuria to start, normal for a week a fortnight later, haematuria again for about 3 weeks, normal for a week, haematuria for 2 days, normal for about 3 weeks, haematuria for about a week, normal for 2 days, haematuria for a day, normal again for a week, haematuria. Body weight. Gained 70 lbs. till the beginning of February, lost rapidly about 80 lbs. in the following fortnight, gained 40 lbs. in the next week and was near its initial body weight.

General condition was rather poor. The animal died suddenly of debility. On P.M. examination its urinary bladder showed numerous papillomatous growths and inflammation towards the blind end, associated with marked thickening of the affected area.

(2) Farm bullock 284 was cured and sent back to farm as a result of treatment with intravenous injection of Formalin ( zii to 120 c.c. of N.S.S.), doses repeated at intervals of 3 to 4 days. No recurrence seen to date, though several years have now elapsed.

(3) H.B. 428. An old standing haematuria case. Urine was positive to the Henzidine test for months, except for being normal for a few days here and there. Normal since 20-2-35. Body weight lost about 40 lbs. during 8 months. General condition was good. Treated with Formalin with practically no effect. Died. Its kidneys presented petechial spots on the surface, and the urinary bladder revealed actively necrotic papillomatous growths.

(4) H.B. 228 was treated with Formalin and Adrenalin. Died of pleuro-pneumonia complicated with acid fast infection. Urinary bladder showed two small papillomatous growths in the mucous membrane. (Acid-fast organisms were found in the smears of the Iliac lymphatic gland).

(5) H.B. 549 Chronic haematuria case. 2 drachms of Formalin in water given from 7-4-38. After 15 days of administration, the quantity of blood in the urine showed decrease. The dose of Formalin was raised to 3 drachms. The decrease con-



tinued to such an extent that one day only a suggestion of blood was seen in the urine. Slight increase from the low level was noticed on 16-6-38, after which there was again a decrease. Treatment was stopped on 24-6-38.

(6) Cow 4/26. Clinical case of haematuria. Intravenously 2 drachms of Formalin in 120 c.c. of N.S.S. on 13-5-38, 16-5-38, 20-5-38. 3 drachms of Formalin in 120 c.c. of N.S.S. on 24-5-38, 30-5-38. No marked change in the condition of the urine. There was a faint suggestion of decrease in the quantity of blood. Treatment was stopped as the cow suddenly showed symptoms of collapse, and died.

(7) H.B. 901, aged 6 years. Commencing on 10-9-34, one capsule containing 5 grms. of Azamine and Emetine Hydrochloride, 2 grs. in 5 c.c. sterile distilled water, intravenously, daily from Sept. 25th to Oct. 4th. Four injections at 4 and 5 days' intervals intramuscularly in alternate buttocks, of 4 grs. in 5 c.c. distilled water about a month later.

Urine. Haematuria to start with, normal for 3 days (20 days after commencement of Azamine treatment and

5 days after Emetine injections), haematuria for a day, normal for about a fortnight, haematuria for a week, normal for a week, haematuria for a day, normal for a week, haematuria for a day, normal for 5 days, haematuria for a fortnight, normal for 16 days, haematuria for a day, normal for a day, haematuria for 4 days, normal for a day, haematuria for a week, normal for 10 days, haematuria for over a month, normal for 3 days, haematuria started again. Bodily condition was good. Body weight lost about 25 lbs. after the intravenous treatment (inappetence and dullness for almost 10 days), then gained about 45 lbs. almost steadily.

(8) H.B. 763. An old standing haematuria case. Inoculated intravenously with 1 in 500 solution of Potassium Permanganate from 12-6-34 to 10-8-34 in doses of 10 c.c., 20 c.c., 30 c.c. and finally 40 c.c. It was ascertained that 10 c.c. of a 1 in 500 solution of the drug had been given to human beings with no untoward results. A start was made by giving the same dose intravenously to the bull, and the treatment was repeated every alternate day. The administration of the drug and the number of injections were controlled according to the results shown. Blood counts and the total quantity of blood cells passed in the urine each day were measured.

H.B. 763.

Date	Pulse	Blood count	Haemoglobin content	Dose of Permanganate
12-6-34	80 per min.	3.98 million	70	10 c.c.
18-6-34	The dose was increased to 20 c.c.			
19-6-34	60 per min.	4.8 million	65	
21-6-34	The injections were repeated.			20 c.c.
24-6-34	" "	" "		No change
				20 c.c.
23-6-34	55 per min.	4.9 million	65	
27-6-34	55 " "	4.2 "	65	
5-7-34	60 " "	5.4 "	65	
13-7-34	60 " "	5.2 "	70	
The haemoglobin content of the blood of this animal remained almost steady at 70% and the erythrocyte count was varied from 5.2 million to 6.0 million during the course of five weekly observations.				
20-7-34		4.4 million	70	
27-7-34	62-65 per min.	5.2 "	70	
3-8-34	63 per min.	5.0 "	70	40 c.c.
10-8-34	63 " "	4.6 "	65	

H.B. 763.

From 12-9-34 onwards, for about a fortnight, Tincture Iodae mitis, zi in 2 lbs. of milk, was administered twice daily. Urine, haematuria all along till a few days after the Iodine treatment was stopped, when it was normal for about a fortnight, after

which haematuria started again. Body weight lost about 25 lbs. Died on 27-12-34.

E.D. Ascites, hydrothorax, tuberculosis and haematuria.

Some inconclusive blood examinations were also carried out.

Methods of prevention and control to be rational must be based upon the life history of the parasite, which in this case has yet to be outlined. The application of lime to land and manuring have come to be recognised as useful prophylactic measures in this disease. The keeping away of animals from rough uncleared areas, drainage of the land, provision of lime water in drinking water, and treating of hay with lime water, are some of the measures which have been reported to be beneficial. Bankier (1936) states that in view of the significance of the pasture conditions in haematuria farms, some Canadian farmers have already made a start at the elimination of surface water from pastures and to prevent their cattle from having access to it.

It will be noted, the conidia and certain other stages of *Aspergilli* have been attributed highly resisting powers. The organism requires moisture, oxygen, and some amount of organic substratum for



growth. Light is perhaps not necessary for growth, and the relative humidity of the atmosphere appears to be more important than the extent of moisture content of the substratum. While growth characters may be affected by varying conditions of the environment, infectivity and vitality of the organism appear to be maintained unchanged. The acid conditions in the soil and water of the haematuria localities reported by several workers, must be favourable to some fungi, particularly members of the genus Aspergillus. It has been found that fungi parasitic on plants require moisture approaching or exceeding saturation point, and that sudden drops in temperature, leading to condensation, are fruitful causes of fungus infections. It has been already shown that these conditions are particularly well represented in haematuria localities.

Various attempts have been made to immunise against fungus infections, including aspergillosis (Sartory and Sartory, 1927, Antivirus of Aspergillus, 1931). Dessy (1932) has gone into the question of the chemotherapy of aspergillosis, and reported that crystal violet, brilliant green and copper chloride in weak solutions are highly chemotherapeutic against Aspergilli. Wedekind & Bruch found that at a concentration of 0.002% perchloride of mercury inhibited Aspergillus growths. More recently Jensen & Orner have

reported that Quinosal 1 in 1,000, and brilliant green 1 in 1,500 are lethal to all species of Aspergilli in 48 hours. It is premature to state to what extent these drugs would be effective in the cure and control of bovine haematuria. Regarding the resistance of the conidia of Aspergilli and their tenacity of life, it may be mentioned that the following figures are available:-

A.glaucus 16 years' quiescence (Hansen)

A.flavescens 8 years but not longer (Hansen)

A.fumigatus 10 years (Eidam)

A.flavus 6 years (Brefeld)

A.oryzae more than 4 years (Wehmer)

A.niger for about 3 years.

Further Aspergillar spores of some strains are known to survive heating to 57°C. for 30 minutes, and dry heat at 110°C. for 30 minutes. Besides the spores of A.niger, A.fumigatus, and A.nidulans can survive exposure to sunlight for 58 hours, or five days of continuous exposure to the intense rays of a summer sun, according to the records of an authority.

In summarising it may be stated that no specific treatment for haematuria has yet been discovered. If the dose of formalin can be controlled according to the state of the disease in an animal it is believed that permanent cures may result, provided of course chances of reinfection are removed. In well established cases

formalin in doses that might be given with impunity to healthy animals, appears to be highly irritant and destructive. In the absence of any specific drug, much can however be accomplished, if the stage and intensity of the disease have not progressed too far. In early mild infections, the cessation of further infection by a change in the environment and the change of the atmospheric and climatic conditions are likely to be highly effective. Iodine salts and copper may act as adjuvants.

## XI. Discussion.

When the facts have to be considered regarding a disease like haematuria, which has baffled many for nearly a century, it must be realised that one must start with a constructive, though not a credulous frame of mind. One must avoid being hypercritical, and look for substantial reasons which may exist to account for past failures. In a case like this, direct experience is always the best guide, whenever a decision about abstruse matters has to be made. To decide whether a particular species or strain of an Aspergillus is the etiological agent of an obscure disease, the universal experience that moulds and even Aspergilli may frequently contaminate bacteriological work need not be underrated. Experience shows that the saprophytic flora of each laboratory frequently varies to a considerable extent. The usual and common contaminants encountered in one may be totally different from those in another. Differences may also exist between particular work rooms. Thus experience accumulates in each laboratory regarding the specific entities which have to be guarded against. At least as far as India is concerned, the writer may state that the commonest related fungus contaminants of cultures are represented by the Aspergillus niger, and the so-called Penicillium glaucum. The recognition of the former by the naked eye is easy due to the enor-



mous size and height of its globular heads, which are more or less widely separated from each other. The three common species of *Aspergillus* encountered there are *A.fumigatus*, *A.nidulans* and *A.niger*. Aspergilli are found growing on soil, tea leaves, in mangoes and other fruits. So far the writer has been unable to find any previous record of the occurrence of *A.flavus* series in India, though the fungus flora of the country appears to have been studied to a large extent.

Regarding the risks of fungus contaminants or chance invaders, it may further be added that the probability of their playing such a role is much less when the fungal organisms are detected in actual histopathological lesions, than when they happen to appear in a culture tube. Further in the case of histopathological tests, there is the additional advantage that pathologists are sufficiently well-equipped with experience of the specific pathological effects in animal tissues of diverse pathogens, and possess knowledge of the subtle distinctions in minute histopathological pictures, to be able to discriminate between secondary invaders of pre-existing disease and primary fungal agents of disease. The investigation of course commences with the finding of the micro-organism in the diseased tissue, but its actual relation is brought out later by considerations such/<sup>as</sup>the microbe being

present in lesions and absent in healthy tissue (leaving carriers out), while being numerous in acute stages, and few in quiescent stages. Besides it may be remembered that even where the rigid requirements of Koch's postulates may not be applied, other equally dependable means of establishing pathogenicity are available, including serological and histological methods, as in the case of glanders, John's Disease and tuberculosis.

In the present instance of haematuria tissues, it must be recalled that past workers have failed to record the occurrence of any fungus. Therefore if a fungal etiology has to be considered, reasonable grounds must be put forward to explain this failure of so many workers. It must be stressed that the undoubted explanation for this lies firstly, in the natural limitation of their methods already dealt with earlier in this treatise,<sup>(pp. 182-6)</sup> and secondly in their failure to employ any of the more appropriate methods applicable to the demonstration of Gram-negative organisms, and of Aspergilli in particular, (like Lactic Acid-cotton blue, caustic potash and glycerine, use of Fleming's fixative or Osmic Acid, Feulgen reaction, use of polarised light).

The next question that has to be answered is:  
Do the outstanding general features of haematuria

conform to those of the other known, mycotic diseases, and particularly of Aspergillosis?

Let this be discussed under (i) the environment, (ii) pathology and (iii) investigational data.

(i) It is wellknown that haematuria has a most striking and limited geographical distribution. How far do mycoses in general behave in a like manner?

To take a few comparable cases, one may refer to what is known about Coccidioidal granuloma, Sporotrichosis and the ordinary ringworms. Ringworms occur endemically in some centres of the population e.g. Tinea cruris of man. Coccidioidal granuloma has quite a limited distribution in Brazil and U.S.A., but the great majority of cases occur in the San Joaquin Valley in California. The reason is not known, but curiously it affects cattle and sheep as well of that area. Similarly Sporotrichosis occurs as a serious disease of man in France, U.S.A. and South America. The majority of cases occur in the Valley of the Missouri River (Reudiger), and in the valleys of Mississippi or its tributaries (Foerster). Increasing numbers of cases have recently been reported from other parts of the world. Equine cases are also stated to be restricted to Missouri Valley. To explain this endemicity, it seems probable that the environmental conditions of the special areas must expose animals or

human beings to extraordinarily heavy infections. The parasite concerned may be prevalent only in the special regions, finding more favourable conditions there, or it may be that these fungi have more tolerance for those endemic conditions than other competing forms. The chances of dissemination of the parasite, and the eventual infection of the population may be facilitated due to reasons of predisposition, crowding, etc..

In haematuria investigations, the suspicion of the influence of the special environmental factors has gradually increased, while the array of different suspected factors has been at the same time narrowed down by the elimination of those that are unimportant. No single environmental factor, or any modification of it has yet come to be specified. The existence of water on pastures, a high rainfall, upland or bench lands, the presence of acidity in soils and vegetation, <sup>some of</sup> ~~are~~ the common features of haematuria farms and localities, about which there appears to be some general agreement. Besides the general observation has been that the disease is mainly restricted to wooded areas and farms which have never been cultivated or which alternatively have been allowed for a long period of time to go out of cultivation.

Australians have found that soils of haematuria farms are particularly rich in plant nutrients, though



apparently weedy.

Are these specific environmental conditions not such as would increase the density of the fungal flora of the locality? Will such conditions as exist in haematuria localities not make the vegetation, forage, bedding and pasture there highly mouldy? The available information shows that moulds, particularly of the type A.flavus usually remain on the surface, <sup>soil</sup> forming a blanket of mycelium bridging over the narrow cracks or openings rather than entering and fruiting deeply in the substratum, and only mycelia being present at a depth of 5 c.m. below the surface of clay soil. Aspergilli are called 'omnivorous', and they can grow freely upon a wide range of substrata. It seems reasonable that A.flavus should thrive under the conditions defined by the common features of haematuria areas in different countries. It is imaginable how surface water would carry the fungus spores due to surface tension, and infect animals.

It has been repeatedly observed and in many countries, that the incidence of haematuria is greatly reduced, or the disease may actually disappear, as a result of cultivation and agricultural improvement of land. If the following data regarding soil microbiology be perused, an explanation for this observation may then be forthcoming:- Hagem found that cultivated

soils had a distinctly different population of Mucorales than pine forest soils. Regarding the influence of the reaction of the soil on the fungus population it was found that a soil receiving manure year after year, in addition to minerals (pH 5.5) had 79,000 fungi; the same soil receiving lime in addition to manure (pH 6.7) had only 10,000 per gram. The soil receiving no manure or fertiliser (pH 5.1) had 87,000 fungi, the same soil limed (pH 7.0) had only 16,000 fungi. The soil receiving Ammonium Sulphate and mineral (pH 4.2) had 129,000 fungi, and the same soil limed (pH 5.2) had 32,000.

Pine forests exist in many haematuria areas, if not in all of them. The above data would appear to apply in a general way to soils of haematuria localities. It seems therefore that the frequent recommendation regarding the cultivation of wooded areas, and liming of land made in the haematuria literature is rational and justified, since both the measures would be directed towards the reduction of the fungus flora of the soil. Moreover it is conceivable that the fungus flora of the soil in a haematuria locality are intimately related to those of the vegetation and the animal body. If the incidence in the soil is reduced, that on the other substrata, the vegetation and the animal body, should likewise be affected. The success

of the above measures of the cultivation and liming of soil in controlling the incidence of haematuria, which has so strikingly been demonstrated in the actual field operations, is therefore more than suggestive of the relation of the fungus flora to the incidence of the disease.

If one endeavours to trace what factors connected with haematuria environments are likely to predispose animals located there to contract fungus infections, one recollects that the relative humidity is high,<sup>and</sup> the temperature is at times very low (with frequent snow-falls and a long cool season at high altitudes). The relative humidity requirements of different fungi vary to a great extent, and the limits within which members of <sup>the</sup> Aspergillus glaucus, A. niger, and A. flavus groups find favourable conditions for growth on animal or vegetable substrata are precise and specific for each. In a general way it may be stated that the majority of fungi require 85-95% relative humidity, and that fungi parasitic on plants need moisture approaching or exceeding the saturation point (Galloway & Burgess). Further sudden drops of temperature leading to condensation, as have been known to be frequent in haematuria areas, have been proved to be a potent factor in aggravating the high humidity conditions in mould spoilage and deterioration of various industrial products.

These two factors may therefore act together and predispose to aspergillar infections of both vegetation and animals. Besides it is known that in all fungi, a favourable temperature, humidity and oxygen supply, determine the rapidity and character of growth, not as individual factors but in their totality. The "hyper-acidity of the soil and vegetation" of haematuria areas, the presence of vegetable humus, and particularly of rich plant nutrients in the soil have been commented upon by several workers. These cultural conditions are recognised as highly favourable for the growth of mould-fungi. The leeching and calcium deficiency of the soil, the deficiency of calcium and manganese in the vegetation have also been observed to be associated with haematuria. It is possible that the deficiency of these two elements, which are known to play a protective role rather than be essential nutrient elements, predispose the vegetation to become fungus-infected. Discussing questions of fungus infections in plant pathology, Jarvis(1932) makes the statement: "Given, then, a favourable host suffering from deranged metabolism which renders it vulnerable, with temperature, humidity and light favourable for germination of spores, and development of mycelium, an epidemic of rust will follow introduction of spores by either natural or artificial means". The essentials of this



statement no doubt apply to the fungus infection in haematuria localities, though the nature of both the infecting and infected are somewhat different. Further Jasevoli demonstrated in 1924 that the nature of the crop cultivated had a bearing on the number of fungi present in the soil, the greatest number occurring in the soil under potatoes, while *Aspergillus* and *Penicillium* were the predominating genera. Haematuria paddocks are 'stale and ferny',<sup>and</sup> the areas are wooded. If the lands are neglected and not cultivated for a length of time, the disease makes its appearance in the special areas. Some animals require a longer time than others in exhibiting the symptom. The time factor appears to represent a richer and denser fungus growth in the area, facilitating a larger and a more closely repeated infections being picked up by the susceptible animals. As a result of long observation, the belief has grown that the disease results somehow from the ingestion of the vegetation and water of the locality. Individual plants like the bracken fern have been incriminated and eliminated. Hence a group of plants or some common factor representing the cultural conditions of the locality, a toxic agent or the presence of some abnormal chemical irritant or the presence of some normal constituent in excessive or deficient amounts has come to be suspected. No chemical agent, toxic or microbial factor has however been traced. The role of the

vegetation and the moist pastures is being determined in the field experiments in Canada. Cannot the Aspergillus sp. be the suspected common factor in the vegetation, in the moist pasture, in the surface water and the toxic agent present in excessive amounts? The cultural examination of certain kinds of local vegetation has yielded cultures of similar and perhaps identical Aspergillus sp., but not in other types of vegetation.

The disease tends to remain localised to the special areas, and does not spread with the transfer of affected animals to stations in the plains. The parasite is no doubt discharged in the excreta of the haematuria cases, but it does not find the cultural conditions required for its rapid multiplication and dissemination there, and the infection of fresh cases does not take place. Again it has been observed by the writer and others that an exacerbation of the disease, and a comparatively rapid death follows the transfer of affected animals to hot stations in the plains. When moved to another free area which is not so hot, it is possible that some early cases may even be cured of the disease. Is it not possible that in the former case the rate of mould growth in the animal's system is stimulated at the higher temperature, <sup>of the place</sup> when <sup>when</sup> and that/in the latter the chances of any further reinfections being

repeated are altogether absent, the recuperative mechanism of the animal body gets the opportunity and time for gradually bringing the pre-existent infection under control. When the land of haematuria farms is brought under cultivation and limed, the climatic and other factors remain the same as previously, but the cultures or flora are profoundly disturbed. The degree of mouldiness of the soil and pasture is thereby reduced. Naturally the amount and density of mould growths that may still be accessible to animals, <sup>and</sup> the frequency and risks of infection through water and forage must be substantially curtailed by those measures. No explanation has yet been advanced as to how both the stall-confined and grazing animals contract the disease. If the fodder is mouldy, the water supply is surcharged with spores, and both types of animals have equal access to them, no immunity of one type of animal or the other could be expected. Without going into any further details of the environment, it may be stated in short, that if one could be sure of the analytical data published by recent workers as applying universally to all haematuria farms and localities, one could imagine of no more favourable aggregation of factors in nature for providing the best cultural conditions for the Aspergillus flavus series, than already exist<sup>ing</sup> in those areas.

(ii) Coming to the pathology of the disease, how far does it fit in with known forms of Aspergill-osis? Is there any uniformity in the symptoms, course, and the lesions of haematuria with those of Aspergillar infections? Contrary to the views held in the literature, the disease is not exclusively a bladder or urinary infection. Other internal organs are affected but the most marked gross lesions are found in the kidney and the liver. There exists ample evidence to show that the extent of the distribution of lesions in forms of Aspergillo-sis is the same, with the most readily recognisable naked eye lesions being exhibited in the kidneys and the liver. Besides in experimental animals, particularly rabbits, the invariable lesions produced by pathogenic Aspergilli have been in these organs. Experimenting with A.fumigatus, Renon made the remarkable observation that fungal elements were passed in the urine, and that actual bladder lesions were produced. Similarly natural cases of Aspergillo-sis of the urinogenital system, producing 'nephromycosis aspergillina', Miller 1891, 'urethritis and vesiculitis', Klausner 1924,<sup>and</sup> reproductive infection (Bendixen and Plum 1929, Jungherr 1935) have been recorded. In the present studies on haematuria, characteristic fungal fructifications have been found inside the blood vessels of certain organs. It appears



that a natural case of vegetations in the vascular system due to aspergilli has also been recorded.

(Salisbury 1875). Schlegel thought haematuria was due to a haematogenous toxin, and Renon believed that in his experimental rabbits, the bladder disease resulted from a venous spread. Jacobson has already emphasised that a common feature of all types of broncho-pulmonary aspergillosis to be the frequent presence of atheromatous lesions throughout the arterial tree of the broncho-pulmonary system. Mucoid cysts of the air sinuses of haematuria cases have their counterpart in the report by Skillern(1927) of aspergillar infection of the air sinuses.

It may be noted that microscopically and essentially, the lesions of aspergillosis and haematuria are the same, being an inflammatory, granulomatous process with haemorrhage and proliferation of cells, associated with the presence of spores and mycelial fragments. The relation of silicosis in the incidence of tuberculosis has received considerable attention. In haematuria as well, the high silica content of the diseased bladder found by Hill, King and Laird, was considered by them to be of significance. They quote evidence to show that silica when slowly dissolved by animal tissues may form organic combinations and the cell cytoplasm may be rich in it.

Turning to the peculiar symptomatology of haematuria, one can for comparison quote Jacobson, who states: One sign that seems suggestive of aspergillosis is the tendency to marked haemorrhages without apparent cause and at intervals from months to years. The haemorrhages are frequently accompanied by no other signs or symptoms of the disease. Another sign of Aspergillosis, which is somewhat suggestive, is that the patients with chronic aspergillosis look fairly well and appear well-nourished in spite of the long duration of the infection. A positive diagnosis can be made only by the finding of the organisms in the sputum, culturally and microscopically.

The identical features of haematuria have been stressed earlier. There exists no explanation for the extreme chronicity, and the most erratically occurring recurrence and intermissions of haematuria. Previous attempts at <sup>the</sup> correlation of these features with age, season, month etc. have failed. Are the facts connected with the life history of the Aspergillus sp., its morphology and function capable of explaining them? The fungus is highly pleomorphic and polymorphic. It responds to the subtle changes in pH, concentration of minerals and nutrients, oxygenation, and other adjustments that are constantly proceeding in the animal's tissues. The fungus is well provided with adaptive

and protective mechanisms to exist under conditions which may be unfavourable in one respect or another.

It seems conceivable that a set of circumstances existing at a time in the animal system regulates the tissue breaking activity of the fungus, or concentrates it in one direction or the other. If the activity is towards the mucosal surfaces, haemorrhages into the urine are the result. Corresponding to each recurrence and intermission of haematuria, the fresh activity in rapid multiplication and tissue breaking of the trophic stages, must be followed irregularly with the quiescence of the exhausted and resistant forms. The relative power of vegetation and reproduction of the fungus must vary with its stage and environment. It is possible therefore that when the delicate hyphal tubes are being slowly extended, the body tissues successfully adjust themselves with the production of more protective tissue, and no surface breach or haemorrhage need take place. The potentiality for the recurrence of haemorrhage, and the chronicity of the disease are therefore represented by the vegetative stage, awaiting the return of favourable conditions. The lumen of blood vessels is enlarged due to the massive increase of the spores and mycelial knots that takes place there, and the vessel wall finally gives way due to the mycelial

activity in it.

The previous workers felt that some factor other than the primary incitant must maintain the lesions in activity in the quiescent stages. Secondary bacterial infections, distomiasis, irritant plants, oxalate crystals with their cutting edges were incriminated. The possibility of cures in early cases, and persistence till death in other cases, on transfer to healthy regions was the subject of similar speculations. These observations can now be explained on the basis of biology of the fungus, and the mechanism as to how these things happen can be demonstrated experimentally. Regarding the course of aspergillosis, Henrici(1930) makes the statement, which is particularly relevant to haematuria: "In a general way the fungus infections are milder and more chronic than bacterial diseases. But they tend to persist for a long time, to spread progressively and eventually to endanger life by metastatic lesions in internal organs".

With regard to the method of infection, aspergillar infections usually take place by the mouth, through the nose, and sometimes by local infections. The role of local vegetation, <sup>and</sup> surface water of haematuria localities has been increasingly suspected. The fungus has been recovered in culture from some local vegetation, and it has produced pathogenic results experimentally. In all probability therefore, haematuria



is due to the ingestion of mould-infected material,<sup>and</sup> the infection is spread through the vascular system to other parts of the body.

Is the *Aspergillus* associated with haematuria, also present in non-haematuria cases? Is it a chance contaminant, accidentally present in the lesions of pre-existing, old disease? The organism has never been cultivated from definitely healthy animals, and its occurrence in India does not appear to have been reported before. An aspergillus was found intimately associated with the essential lesions of the disease in several places, which were as distant as Canada, Ireland and the north and south of India. The contaminants of pathological material in one or several countries cannot invariably be an aspergillus of the same group of classification. The writer has had very extensive experience of studying numerous sections of tissues from many chronic diseases of bacterial and helminthic origin, but has never encountered aspergilli as contaminating other pathological lesions. The fructification of aspergillar heads inside the vascular system, the isolation in pure culture of the fungus from the jugular blood and from the heart, and the successful pathogenicity tests are unusual findings, never ascribable to contaminants. The same or similar aspergillus exists on some of the local veget-

ation. A number of fungi which grow free in nature can produce animal disease, and the existence of the fungus in the locality need mean no more than the source from which infection is to be expected. However there exist two criteria by which pathogenic and non-pathogenic aspergilli can be differentiated fairly definitely. Organisms capable of producing internal mycosis produce optimum growths at  $37^{\circ}\text{C}$ ., but the other method available is the pathogenicity test in experimental animals, and is the more dependable method. The animal of choice is the rabbit, and the method of administration is the intravenous. The haemat-  
uria aspergillus has fulfilled both the criteria. It may however be mentioned in passing that it is sometimes extremely difficult to set up lesions artificially with even pathogenic species of fungi, as in the instance of actinomycosis. Workers who have attempted transmission experiments with various types of ordinary mould fungi collected from nature have been struck with the somewhat consistent failures to do so. The exceptions which exist are notably few.

(iii) There are some data which have emerged from previous investigations, and should be explained or interpreted here. The Australian workers, while attempting to trace any poison or chemical deficiency in the pasture by analytical methods, suggested the

"possibility of a low protein intake and possibly of a low sulphate intake", as a result of urine examination. From the knowledge of the requirements of these nutrients in aspergillar metabolism, it is possible that the recorded subnormal excretion of these is indicative of the infection. This awaits elucidation. One of the most important observations, and one which was believed to be the proof of Hadwen's theory, relates to the presence of calcium oxalate crystals in the urine and the bladder. On the basis of this finding oxalic acid bearing plants were incriminated. The theory has now been overthrown, but no explanation has been offered, or any importance attached to that original observation, though they have been met with by others. Hadwen stated that these crystals with sharp cutting edges were present in their largest numbers, only in the early stages of haematuria. In the present writer's experience, cultures of the haematuria aspergillus have been observed to produce the largest crop of oxalate crystals in young cultures only. The explanation of the crystals is therefore the aspergillus infection. The oxalic acid bearing plant of Hadwen, searched for in vain previously, turns out to be the aspergillus. The suspicion of the present writer of an endogenous production of oxalic acid, expressed in 1931, is confirmed. It remains now to consider

the findings of some peculiar structures by a group of investigators in different countries, and to see if any explanation can be advanced for them.

Roger described some structures as "several layers round a peduncle in the manner of petals of a flower". It is difficult to conceive of any animal tissues fitting in with this description, while it would just express the appearance of an aspergillar head. Bankier discovered some 'large cells' in the urine, but could not offer any opinion regarding their identity. Other workers, (Goetz, Case, Scharer, Roger, p.62, p.63, p.66, p.64 respectively of this Thesis), have also found such large bodies, either vacuolated or capsulated, but have hesitated to indicate their exact nature. Some were inclined to pass them off as degenerated epithelial cell, while others believed that they were coccidia.

In view of the fact that these have been interpreted as coccidia so many times, and even very recently, it is necessary to give some detailed consideration to the possibility to eliminate it once for all. To prove any obscure disease to be a form of Coccidiosis, it is important to ascertain first what, if any, coccidia are normally present in healthy animals in the locality. Any work attempting to establish a coccidial origin can have no scientific value till the chances of contamination with the well-known "carrier



infection" have first been eliminated. Eimeria zurni is known to be widespread in India, and the probability of extraneous contamination with these organisms, both in male and female animals alike, is considerable. It may be added that out of 855 heads of apparently normal cattle autopsied in 1922-24, Cooper failed to demonstrate coccidia in as few as 1% of the cattle only. In determining whether a particular species is pathogenic or not, it is important to know the nature and the developmental stage of the coccidial oocyst. Pathogenicity tests with any coccidia recovered from haematuria cases were not carried out by previous workers. Coccidia may be found in wet faecal preparations, but no coccidial theory can be entertained till precise details have been put forward of the character of division inside the oocyst (the only differential criterion among the genera of Coccidia), and of the stage of development attained by the parasite, indicating whether any clinical significance exists. Though this theory received support from more workers than has been the lot of any<sup>other</sup>, it has never been considered seriously by experts. The reason for this may be that all the existing knowledge is altogether against such a possibility. For instance, the morbid anatomy and minute histology of the various forms of coccidiosis are all known, and these bear no resemblance to those

of bovine haematuria. Coccidial invasion in mammals is restricted to the intestines and liver, and such an invasion of the genito-urinary tract (excepting in birds) would be most unusual. The histological features of Coccidiosis are typical, being characterised by what may be called an inter-canalicular papillo-adenoma, and the rapidly multiplying oocysts are readily detected therein. Haematuria lesions are dissimilar, consisting of simple papilloma, mucoid cysts and angioma, associated with a tendency to simulate malignancy (never seen in coccidiosis). Oocysts have never been detected in haematuria lesions by experts on those organisms. Clinically, haematuria is always progressive, though at times very mildly, but a coccidial infection may lie dormant in an animal, being never resuscitated. Coccidiosis in India occurs in both the plains and the hills, probably equally; haematuria, on the other hand, is restricted to elevated land and hilly wooded regions.

The present investigation has yielded, from cultural and experimental studies, ample evidence that the obscure structures described by previous workers and repeatedly found by the present writer, represent different stages of development of the aspergillus concerned, and are not coccidia.

It is now necessary to consider some of the other

investigational data recorded by previous workers with a view to find an explanation why the *Aspergillus* should be localised in the urinary tract, and particularly in the bladder. Is it because these organs happen to be the last stages in the excretory system of the animal? Or is it because of the special composition of the contained urine and its salts that the fungus markedly localises there? Firstly to compare haematuria lesions with those of another mycotic infection of the urinary tract, one may quote Jacobsen, who states "Actinomycosis of the urinary tract is protean in its manifestations and may simulate a bacterial pyelonephritis, cystitis, a hypernephroma, carcinoma, sarcoma etc". No comments are necessary on this statement. As a result of quantitative and qualitative estimations of the urine of normal and haematuria cattle, striking differences were revealed by Australian workers. Urine samples of cattle from haematuria farms showed a considerable diminution in the amounts of total nitrogen, urea, creatinine and sulphur, associated with a large increase in the calcium, oxalic acid and combined phenols. It is known from published data that large quantities of total nitrogen can be metabolised by *Aspergilli* and urea can be an excellent source for this purpose. Besides, these organisms can utilise sulphur from both organic and inorganic combinations. Urine was one of the orig-

inal cultural media used by Pasteur and this has been employed by others for growing various organisms, particularly fungi.. It appears that the mineral salts and proteins present in urine are such as would satisfy the cultural requirements of Aspergilli. The presence of excessive amounts of phenols, oxalates and calcium in haematuria urine would be expected as metabolic products of A.flavus. The ferric chloride test to which the fungus responds is essentially a phenolic reaction. One would expect normally that the effect of the fungal activity in the urinary bladder would be capable of confirmation by chemical methods. The presence of excessive amounts of silica in the affected bladders as well as in the vegetation of haematuria farms has been commented upon earlier, but it is necessary to refer to it again in connection with the statement of Raulin that the ash of fungo-cellulose consists almost exclusively of silica, it being very useful for strengthening the fungal membrane. Besides he includes silica in the list of foods essential for fungi.

Regarding the mechanism of localisation, one may recall that the various fungal organisms known to occur in the urogenital system and producing tubercle-like lesions, such as actinomyces, leptothrix, aspergilli, and yeasts, have repeatedly been studied, but no clear explanation has yet been offered. Workers (Grawitz,



Renon) found that when fungi were injected intravenously the mould spores appeared in the urine. In this connection the report by Rosenow and co-workers (1922, Jl. Lab. & Clin. Med. 7, 707-722) of the successful transmission of various urinogenital infections in animals, by means of intravenous administration of organisms obtained from the tonsils, nasal sinuses, dental abscesses of persons affected with urinogenital affections, is of interest. Again David and McGill in 1923 were able to produce urinary infections in dogs by damaging or obstructing the intestine and found that the colon bacillus was present in the blood of some of these animals. It is not necessary to generalise from these data of the bacteriological field, but the analogies should certainly point to lines along which further investigation may be directed.

It has already been stressed earlier that systematic grouping has no relation to pathogenicity, and that various members of a group may behave differently in this matter. Some members of the A.flavus group are cosmopolitan, but that does not necessarily imply that any other members should not be pathogenic. A.flavus series is very widely distributed in some places, and bovine haematuria is not known to occur in a number of them. These facts do not militate against bovine haematuria being a form of Aspergillosis due to a member of Aspergillus flavus series.

## XII. SUMMARY AND CONCLUSION.

The geographical distribution of chronic bovine haematuria in widely separated and specially restricted areas in many countries of the world has been studied and marked on maps. The dates of the first records from each country have been considered with a view to ascertain if there exists any evidence of the introduction of the disease from one country into another. Contrary to the statements of workers, it has been shown that the disease existed for over a century in some European countries. A comprehensive survey of the previous investigations has been made for the first time. The recorded results have been summarised and an attempt made to correlate the definite facts which emerged from previous studies. A satisfactory explanation for the many hitherto inexplicable data has been put forward. A large amount of altogether new ground has been broken. All the possible factors imaginable as connected with the specific enzootic areas have been analysed in great detail. The theories of causation of the disease put forward during the last century have been reviewed. The trend of the present day researches on the obscure etiology as recorded in the latest publications up to date from Europe, Australia, Canada and U.S.A. has been indicated. It has been shown that present investigations are being concentrat-

ed in the belief that a chemical deficiency or imbalance is at the root of the trouble. The absence of any dependable working hypothesis having the prospect of effecting any substantial elucidation has been repeatedly commented upon even to the present day. The only rational measure in dealing with affected animals has been, according to workers with extensive experience, the destruction for meat.

The aim of the present investigation has been to elucidate the obscure etiology and thereafter to evolve rational lines of cure and control of the disease. The problem has been one of animal pathology but considerable incursions into the domain of mycology were required for the understanding of the biology of the mould fungus in the animal system, particularly to recognise and differentiate between the uninucleated stages of the parasite from the tissue cells, as also to pick out true fungal elements inside the animal tissue. The writer's researches were not confined to the disease as it occurs in India but included morbid materials for comparative studies from Australia, Canada, Formosa and Ireland. The disease in India has been studied at the seats and also in the laboratory. The actual localities have thus been compared with those of other countries. Clinical cases were kept under observation, and one animal was studied for as long as eight years. Samples of faeces, blood smears, temper-

ature, body weights and urine samples were examined as a routine, besides when any animal died full opportunity was taken to study the lesions in fresh and preserved materials. Cultural examinations were carried out firstly for bacteria, secondly for protozoa and finally for fungi. The methods of examination employed by previous workers were tested out, and staining methods were modified in several ways in the hope that although the histological structures may lose in staining capacities the features of the parasite may stand out more prominently. Recourse was taken of purely mycological methods of tissue fixation and staining, standard cultural media, and special experimental studies were made in sections of vegetable and animal tissues to determine the morphological features of the parasite under the conditions existing inside the tissue. Pure cultures of an *Aspergillus* were constantly obtained from the heart blood, bladder and kidney tissues, and from urinary sediments of haematuria subjects. The cultures conformed very closely to each other in their chromogenic and colony characters, in their capability to ferment certain sugars and not others, in killing rabbits and other laboratory animals with constant lesions more markedly localised in the kidneys, liver and the bladder. The fungus cultures were found to grow best at 37°C., a feature common among pathogenic fungi. By the intravenous administration of the pure fungus



culture, a healthy bull was killed and the lesions produced were extremely similar to those characterising the natural disease. Similarly with urinary sediments from haematuria animals being given subcutaneously or orally actual haematuria or degrees of definite bladder disease ~~were~~ set up. From the heart blood and from internal organs of these cases of successful transmission the identical fungus was recovered back, besides the intimate association of the fungus to the exclusion of any other organism in the lesions was proved not only in these natural cases and experimental subjects. The Koch's postulates were thus found to apply to these experiments. Microscopically the fungus in the tissues from as widely separated places as Canada, Ireland and the North and South of India was found to be characterised by the same typical conidiophore, ~~and~~ conidia, and the perithecia from the Canadian material agreed in morphology and measurements with those in the Indian disease. It was remarkable that by the use of polarised light and staining methods tried successively, a striking picture of conidiophoric fructification inside the blood vessels of internal organs of both natural cases and experimental cattle was revealed. This finding by itself suggests a very significant parasite-host relationship between haematuria and the *Aspergillus*.

Any finding to be accepted as of etiological sig-

nificance must indicate that sufficiently elaborate precautions had been taken to eliminate contaminants, and secondary invaders of already diseased tissue. The organism was never cultivated from any definitely healthy animal, its occurrence in India was not reported before, besides an *Aspergillus* would not be associated with material from different sources, nor would pure cultures be obtained from the jugular blood of natural cases. The successful pathogenicity test, and the fact that such *Aspergillar* fructifications have never been encountered in pathological lesions of the several chronic diseases, of which the writer has had the opportunity of examining hundreds of sections, are, it would appear, sufficient proofs of the etiological role of the *Aspergillus*. Moreover the new finding must explain all the hitherto inexplicable but definite data already recorded by others, in connection with the climatic and environmental influences, pathological and clinical features, together with other investigational data. The high humidity conditions, frequency of water condensations, the presence of humus and rich plant nutrients in the soil, the acidity and moistness of the soil, the deficiency of certain minerals in the vegetation, the suspicion of oxalic acid bearing plant as the cause of the disease, the finding of oxalates in the diseased bladder and urine, the repeated description of coccidia-like, epithelial-like,

and reports of half-moon bodies, Roger's florette, are all interpretable as associated with the life history of the *Aspergillus*. Further the excretion in the urine of excessive quantities of phenols, oxalates and calcium, the presence of large quantities of silica in the bladder and urine, the diminished excretion in the urine of certain other ingredients can be accounted for as being due to the *Aspergillar* metabolism in the animal tissue. The failure of the previous workers to record the presence of the Gram-negative organism, the failure to differentiate the uninucleated stages of the fungus from blood corpuscles in the tissues, unsuccessful transmission experiments with large amounts of fluid urine, blood and bladder lesions have been explained. The methods employed by them were limited in scope and more appropriate methods of staining, experimental transmission, diagnosis of the disease in its earliest stages have been indicated. It has been shown how the environmental factors, the disease producing *Aspergillus* and a constitutional predisposition of subjects may co-operate together to set up the disease entity. It has been shown how haematuria closely conforms to the manifestations in clinical symptoms and pathological lesions to the features of *Aspergill*-osis. The tendency to recurrent and marked haemorrhages in an otherwise well-nourished subject, the long duration and comparatively stationary symptoms, the

polypoid or papillomatous inflammatory growth, which characterise several well-known mycotic infections, have been shown to be equally associated with both haematuria and Aspergillosis.

The cultures of the *Aspergillus* from haematuria cases have conformed very closely in structure, morphological details, biochemical characters and other properties and have been placed in the A. flavus series.

In conclusion it may be noted that the investigation has covered over eighty natural cases of the disease from different parts of the world. All the facts regarding the disease including the inherent difficulties associated with the study of the disease have been surmounted and further fields for investigation opened up. The problem has been unfolded step by step. Sufficient clear data has been put forward to make the conclusions irresistible. The final acceptance of any scientific discovery depends upon the observations being eventually confirmed by a number of other workers. The readiest way of testing the truth is to apply lactic acid and cotton blue or caustic potash to fresh diseased tissue, or to apply the special staining and fixing of tissues, together with recourse to polarised light.



# REFERENCES.

- Abbot, E.V. (1923) The occurrence and action of fungi on soils.  
Rev. App. Mycol.
- Adami (1917) Some cases of Haematuria in bovines. Clinica Vet. 40, 611.
- Alexander, R.A. (1938) Report First Imp. Vet. Conference, London, p. 79.
- Allardyce, J., Fleming, R.H., Fowler, F.L., and Clark, R.H. (1930). Blood Normals of cattle -- Some pathological values. Canad. Jl. Res. 3, 120-124.
- Anacher Cited by Hutyra & Marek (1938)
- Anderson (1842) A singular disease in a cow. Veterinarian 15, 27-30.
- Andersen, J.A. (1918) Malignant haematuria of cattle. Maandeskr. f. Dyr1. 30, 580.  
Abst. Schweiz. Archiv. f. Tierh1k. (1919) 61, 310.
- Angeloff, St. (1910) Ueber ein im Rhodopigebirge (Bulgarien) vorkommendes Blutharnen des Rindes (Haematuria vesicalis bovis rodopensis). Arch f. Wiss. u. Prakt. Tierh1k. 36. (supple.), pp. 670-675.
- Armstrong, G.M. (1922) Studies on the physiology of the fungi -- sulphur nutrition -- the

- use of thiosulphates as influenced by H. ion concentration.
- Ann. Mo. Bot. Gard. viii, (3), 237-281.
- Arnold, (1890) Das Stallrot und seine behandlung. Lydtins tierartzliche Mitteilungen No. 3, 33-38.
- Aruch (1900) Malattie del sistema nervosoe dell'apparato genito-urinario p.256. Milano.
- Australia (1938) 12th. Ann. Rip. Coun. Sci. & Indust. Res. p.29. (1930) 4th Ibid.
- Avril (1929) Contribution a l'etude de la cystite hemorrhagique du batail. These de doctorat. Paris.
- Bendixen, H.chr., and Plum, N. (1929) Schimmelpilze (Aspergillus fumigatus und Absidia ramosa) als Abortsursache beim Rinde. Acta. Path. Microbio Scand. 6, 252-322.
- Bhagwat, Singhjee (1896) A short history of Aryan Medical Science. London. 1896.
- Birkinshaw, J.H. (1937) Biochemistry of the lower fungi. Bio. Rev. 12, 357.
- Bernton, H.S. (1930) Jl. Am. Med. Ass. 95, 189.
- Bankier, J.C. (1936) Progress report on investigation of bovine haematuria.

Ann. Rep. Vet. Dir. Gen. Dep. Agri.  
Canada pp. 47-50.

Blakeslee, A.F. and Gartner, R.A. (1915) Reaction of rabbits to intravenous injections of mould spores. Biochem Bull. 4, 45-51.

----- (1913) On the occurrence of a toxin in juice expressed from the bread mould - Rhizopus nigricans.  
Ibid, 2, 542.

Blochwitz, A. (1929) Die Aspergillaceen system und Phylogenie.

Ann. Mycol. 27, 185-204, 204-240.

----- (1928) Berlin. Deutsch. Bot. Gesellschaft. 46, 516-524

Boudeaud (1893) A propos de l'hématurie des betes bovines.

Bull. Soc. Cent. de Med. Vet. p.86.

Brown, J.H. and Brenn, L. (1931) Method of the differential staining of Grampositive and Gramnegative bacteria in tissue sections. Bull. John Hopkins Hosp. 48, 69.

Bull, L.B. (1929) Poisoning of sheep with soursobs. Austr. Vet. Jl.

----- with Dickinson, C.G. (1929) Enzootic Haematuria of bovines. Jl. Counc. Sci.

- and Indust. Res. 2, 232-238.
- with Dickinson, C.G. and Dann, A.T. (1932)  
 Enzootic Haematuria (Haematuria  
vessicalis) of cattle. Counc. Sci.  
and Indust. Res. Pamphlet No.33.
- Butozan, V. (1935) Abst. Vet. Bull. (1938) Hae-  
maturia Vesicalis in Yugoslavia.  
 Veter. Archiv. 8, 96-100. (1938) Ob-  
 servations on cases of chronic hae-  
 maturia in Yugoslavia. Ibid, 8, 158-  
 169.
- & Tramer (1937) Calcium and chlorine oinsin  
 serum and urine. Ibid, 7, 371-376.
- Cadeac, M. (1913) Cystite chronique hemorrhagique.  
Jl. de Med. Vet. 65, 278-288.
- Canada (1938) Ann. Rep. Vet. Dir. Gen. p.41.  
 (1930) Ibid. p.40.
- Carlens (1923) Haemoglobinuri hos notkreatur.  
Svensk. Vet. Tidskr. 28, 199-203.  
 Abst. Berlin. Tier. Woch. 40, 163.
- Case, L.N. (1911) Red water in cattle. Rep. Bd.  
of Commiss. Agri. Forest. Hawaii.  
 pp. 199-204.
- Cavallazzi (1889) Sulle affezioni ematurio car-  
 bonchio ematico, febbre semplice domi-



nanti nei bovini di Gavardo, Sopraponte, ecc.

Clinica Vet., cited by Giovine (1933).

Challenger, Klein and Walker (1929) Jl. Chem. Soc., 1498.

Chaudhuri, H. (1933) Formation anormale de conidiophores chez l'*Aspergillus nidulans*. Bull. Soc. Bot. France 80, 29-30.

Chieri & Frohlich (1911) Jl. Chem. Soc. 100, Pt. 2, 1018.

Church, M.B. & Buckley, J.S. (1923) Laboratory feeding of molds to animals. North Amer. Vet. 4, 7-13.

Cleemann, J.H. (1918) On haematuria of cattle of the autumn season of 1918. Maandeskr. f. Dyr 1. 30, Heft 19, 548. Abst. Schweiz Archiv. f. Tierhkl. 1919, 61, 308.

----- (1923) Til sporgs maalet on Haemoglobinurien hos malkekvoget. Abst. Berlin. Tier. Woch. 40, 163.

Cleland, J. Burton (1911) Endemic Haematuria in cattle. Jl. Trop. Vet. Sc. 6, 125.

Clutterbuck, P.W. (1936) Recent developments in the biochemistry of moulds. Jl. Soc. Chem. Indust. 55, 55-61.

- Cocheril, J. (1930) Contribution a l'etude des haematuries toxiques chez les bovins. (Vergiftung durch Bingelkrank) Inaug. Diss Alfort. Abst. Jahreb. Vet. 1931, 1, 597.
- Craig, J.F. (1930) Chronic Haematuria of cattle. Vet. Rec. 10, 68-71, 87-88?
- & Kehoe, D. (1921) Common sorrel. Investigations as to the poisonous nature. Jl. Comp. Path. & Therap. 34, 27.
- (1923) Chronic haematuria of cattle in Ireland. Jl. Dept. Agri. & Tech. Instr. Irel., 22, pp. 375-377.
- (1925) Chronic haematuria in cattle. Ibid, 25, 211-214.
- Cruzel Maladies de l'espece bovine. p. 562.  
cited by Avril (1929)
- Dale (1909) On the morphology and cytology of A. repens. Ann. Mycol. 1, 215-225.
- Dann, A.T. ( ) Chemical composition of the urine of cows. Austral. Jl. Exp. Biol. & Med. Sc. 53
- Datta, S. (1932) A new fungus Eidamella actoni parasitic to the dog. Ind Vet. Jl. 8, 1-10. Abst. Rev. App. Myc. 1932, 11, 716.
- (1931) Ind. Vet. Jl. 8

- (1936) An. Rep. Imp. Inst. Vet. Res.  
Muktesar pp.26-27.
- (1932) The etiology of Bovine Nasal  
granuloma. Ind. Jl. Vet. Sc. Ani.  
Husb. 2, 131-140.
- (1933) The etiology of Bursati. Ibid,  
3, 317-236.
- (1934) The etiology of Enzootic Bov-  
ine Haematuria, Part I. Ibid, 4,  
341-361 Plates XIX - XXIX(a). Abst.  
Lancet, 228, 1511-1512.
- (1938) Bovine Maematuria. Report  
First Imp. Vet. Conference, London.  
pp. 78-81.
- D'Autilio (1891) Di una specie di ematuria  
(piscias angue) nei bovini. Il Moder-  
no Zooiatio. p.125. Cited by Giovine  
(1933).
- De Filippis (1891) Ematuria enzootica dei bovini.  
Giornale di med. vet. pratica. p.200.
- Delcroix (1905) De l'influence de l'etat de  
gestation sur l'apparition de l'hém-  
aturia chronique des bovides. Ann. de  
Med. Vet. 54, 244-5. Also in --  
Progres Veter. Fasc, 12. Abst. Jahreb.  
Vet. 1905, p.202.
- Dessy, G. (1932) The chemotherapy of mycoses-

- Aspergillosis. Experiments in vivo.  
Boll. Sez. Ital. della Soc. Internaz.  
Microbiol. Abst. Rev. App. Mycol.  
 1932, 11, 715.
- Detroye (1891) Recherches sur d'ematurie  
 essentielle du boeuf. Bull. Soc.  
Centr. Med. Vet. P.512.
- (1904) L'hematurie chez les bovides.  
Ibidem p. 110.
- (1904) Nouvelles recherches sur la  
 nature et la pathogenie de l'hematurie  
 essentielle du boeuf. Ibidem p. 187.
- (1904) Sur l'hematurie des bovides.  
Ibidem p. 442.
- Dickinson, C. and Bull, L. (1928) Haematuria of  
 cattle at Mount Gambier. Jl. Counc.  
Sci. Industr. Res. Austr. 1, No.3.
- (1929) Enzootic haematuria of bovines.  
 Some preliminary observations on the  
 investigation. Ibid. 2, 232.
- Downham, K.D. (1928) Bovine Haematuria. Report of  
First Imp. Vet. Conference. Ldn. p.80.
- Drouard, M. (1838) Veterinarian, p. 130.
- Dumas (1934) Lyons Thesis. Abst. Vet. Bull.  
 309.
- Durin, G. and Unglass, G. (1931) L'hematurie essent-  
 ielle des bovides. Rev. de Path.



- Compar. et d'hygiene generale. Oct.
- Eckles, C.H., Fitch, C.P. and Seal, J.L. (1924)  
Molds in silage and their significance  
in the production of disease in live-  
stock. Jl. Amer. Vet. Med. Ass. 64,  
(n.s.17) 716-722.
- Falck, R. and co-worker (1924) Rev. App. Mycol. 1925,  
4, 52.
- Fleming, R., Fowler, F. and Clark, R. (1930) Hae-  
maturia vesicalis. Canad. Jl. Res.  
3, 125-129. Abst. Biol. Abstracts.  
1931, 5, 1698.
- Fraser & Chambers (1907) The morphology of A. herb-  
ariorum. Ann. Mycol. 5, 419-431.
- Frenkel (1931) Pneumonia Aspergillaire du  
cheval. Rec. de Med. Vet. 107, 417.
- Friedbergher & Frohner Pathologie speciale, 1, 202.  
Cited by Avril (1929).
- Friedmann, T.E. (1934) Science, 80, 34.
- Fujemaki et al (1927) Gana, 21, 9.
- Funke, G.L. (1929) On the heredity of some char-  
acteristics of two strains of Asp.  
flavus-Oryzae. Rec. Trav. Bot. Neerl.  
26, 14.
- Galloway, L.D. (1936) Ind. Jl. Agri. Sci. 578-585.
- Galtier and Boudeau (1886) Ueber Blutharnen des  
Rindes. Rec. Bull.

- Galtier (1892) Recherches sur l'hématurie  
essentielle des animaux bovins. 1:  
note. Bull. Soc. Centr. Med. Vet., 718.
- (1893) Recherches sur l'hématurie  
essentielle ou cystite hémorragique  
des animaux bovins. Ibidem, 2: note,  
p. 90.
- Ghosh, S. (1933) Ann. Rep. Calcutta School of  
Trop. Med. p.83.
- Gibson, A.G. (1930) The mycoses of the spleen.  
London. pp. 169. 10pls.
- Gibson, Joan (1934) On the nature of clubs in  
actinomycosis. Jl. Path. and Bact.  
39, 533-5.
- Giovine, D. (1933) L'ematuria dei bovini da  
Cystite chronica emorragica vegetante.  
Clin. Vet. 56, 169-179. Abst. Vet.  
Bull. 3, 33, 722. Jahreb. Vet. p.121.
- Glockner, E.E. (1913) Haematurie bei Rindern.  
Berlin. Veterinarn. Sachsen. pp. 95.
- Glockner, E. and Haupt, H. (1922) Beitrag zum  
Stallrot des Rindes. Munch. Tier.  
Woch., 73, 161. Abst. Jahresb. vet.  
med., 41-42, 215.
- Gmelin (1897) Uber Stallrot. Deuts. Tier.  
Woch, 5, 212.
- Goetz, H. (1906) Beitrage zur Pathologie der

- Cystite verrucosa des Rindes. Schweiz. Arch. f. Tierhkl. 48, 1-31. Abst. Vet. News. 1907, 4.
- Gottardi (1935) Profilassi, 8, 25-26. Abst. Vet. Bull. 1936. 206.
- Grauer, F.W. (1930) Haematuria vesicalis. Thesis. University Brit. Columbia. Sept.
- Greenbaum, S.S. (1922) On the biologic properties of pathogenic moulds. Jl. Infect. Dis. 31, 26-31.
- Hadwen, S. (1912) Haematuria. Rep. Vet. Dir. Gen. Canad. p.92.
- (1913) Dept. Agri. Ibid. pp. 74-81.
- (1914) A study of haematuria in France and other countries. Ibid. pp. 119-129.
- The nature of food cattle eat when wandering at large in the forest of British Columbia. (Haematuria) Ibid. p. 122.
- (1914) Protocol of experiment to prove that calcium oxalate crystals are capable of injuring the bladder and producing Haematuria. Ibid. p.124.
- (1915) Bovine Haematuria. Jl. Amer. Vet. Med. Ass. 51, 822-830. Abst. Trop. Vet. Bull. 1917, 5, 267-269.

- (1916) 1. Rep. Vet. Dir. Gen. Abst.  
Rec. de Med. vet.
- (1916) Landw. Jl. Bayern. 6, 183.  
Munch. Tier. Woch. p. 829.
- (1917-18) Munch. Tier. Woch. p.81,  
p.287.
- (1921) Jl. Amer. Vet. Med. Ass. 51,  
822. Abst. Jahreb. vet. med. 1921,  
p. 215.
- Hansen (1926) Single spore culture. Science,  
64, 384.
- Henrard, P. (1934) Polarity, heredity and vari-  
ation in different species of Asper-  
gillus. Cellule, 43, 3, pp. 351-426.
- Henrici, A.T. (1914) Jl. Med. Res. 30. (25 ns.),  
409-415.
- (1930) Molds, yeasts and Actinomycet-  
es. pp. 104-5. New York.
- (1939) Endotoxin from A. fumigatus.  
J. Immunol. 36, 319-338.
- Herat & Unglas (1932) Sur la prophylaxie de l'ematurie  
essentielle bovine. Rev. Path.  
comp. et l'hygiene gener. p. 571.
- Highman, U.J. (1930) Jl. Am. Med. Ass. 95, 1158.
- Hill, W.H., King, H.M. and Laird, D.G. (1933) Some  
further studies on the etiology of  
bovine Haematuria vesicalis (Red



- water) in British Columbia. Sci. Agri. Ottawa. 13, 545-560.
- Hink (1886) Ueber Stall und Weidroth des Rindviehes. Lydtin's tier. Mitth. 22, p.183. Abst. Jahreb. Vet. p. 124.
- Hippmann (1932) Berlin. Tier. Woch. 25, 205.
- Holmes, J.D.E. (1914) Bursati. Mem. Dep. Agri. Ind. Vet. Ser. 2, No.5.
- Holtz, H.F. (1930) Effect of Calcium and phosphorus content of various soil series of western Washington upon the calcium phosphorus of oats, red clover and white clover. Wash. Agri. Expt. Sta. Bull. No.243, 1-45.
- Hopkins, S.J. and Chibnall, A.C. (1932) Growth of Asp. versicolor on the higher paraffins. Biochem. Jl. 26, pp. 133-142.
- Hubner (1842) Ueber Blutharnen beim Rind. Rec. de. med. vet. 19, 173.
- Hutyra & Marek (1938) Special Pathology and Therapeutics of the disease of domestic animals. London. Vol. 3, 3-5.
- Ichikawa, K. (1921) Ueber die Endemische Haematurie bei Rindern mit Bhasentumor in der Schinchikn-egend auf der Insel Formosa, mit besonderer Beruckrichtung des bei diesem Fallen entdeck-

- ten Schistosomum. II. Mitteilungen.  
Ni. Byor. Gak. K. Tokyo. 2, 460-2.  
 Abst. Jap. Jl. Med. Sci. 1922, 1, 63.  
Trop. Vet. Bull. 1924, 12, 20.
- Inchley (1921) J. Pharm. & Exp. Therapy, 18,  
 237.
- Jacobson, H.P. (1932) Fungous Diseases. Ldn. p.276.
- Jacoby & Friedel (1933) Ca/Mg ratio of serum in oxalic  
 acid poisoning. Biochem. Ztschft. 260,  
 451-453.
- Jaffe, R.H. and Hill, L.R. (1928) Fungus Diseases.  
 Illinois.
- Jasevoli (1924) Arch. Path. 6, 196. Rev. App.  
Mycol.
- Jensen, V. and Orner, H. (1935) Investigations on  
 the applicability of certain substances  
 for the preservation of medicaments.  
 Moulds and the like. Rev. App. Mycol. 14, 115.
- Jones & Little (1931) Cystite et pyelonephrite des  
 bovides. Rec. de Med. Vet. 107, 100.
- Jouquan (1885) Rec. Med. Vet. 686. Cited by  
 Avril (1929).
- Jungherr (1935) Mycotic affections of the  
 bovine reproductive system. Jl. Amer. Vet. Med. Ass., 86, 64-75.
- Kalkins, J.W. (1924) Bull. West. Washing. Exp. Sta.

- 12, 3.
- Kalkus, J. (1913) A preliminary report on the investigation of bovine red water (cystitis). W. Wash. Exp. Sta. Bull. No. 112, pp. 1-27.
- (1931) Bovine Red Water. Cystic Haematuria. Vet. Med. 26, 476-81.
- Kalkus & Sawyer (1924) Red Water in cattle. pp. 53. State College of Wash. Agri. Exp. Sta.
- Kearney, W. (1917-18) Haematuria. Ann. Rep. Dept. Agri. Br. East Africa. pp. 176-7.
- Kerrigan, J. (1926) Chronic haematuria affecting cattle. N.Z. Jl. Agri. 33, 85-87.
- Klausner, E. (1924) Ueber ein noch nicht beobachtete Infektion der Schleimhaut des Urogenital System (durch Hefepilze?) urethritis, cystitis, vesiculitis haemorrhagic. Derm. Woch. 78, 342-3.
- Klobouk, A. (1931) Haematurie oder verrucose Zystitis der Rinder. (Given in Fortbildungskurses, Veterinary College, Brno, Dec. 1930). Prag. tierarztl. Arch. 11, Pt. B, 10.
- Klotz, L.J. (1923) Studies in the physiology of fungi. XVI. Some Aspects of nitrogen metabolism of fungi. Ann. Missouri Bot. Gard. X, 4, pp. 299-368.

- Kotliar, E. (1894) Contribution a l'etude de la pseudotuberculose aspergillaire.  
Ann. Inst. Past. 8. 479-489.
- Krishnamurti Ayyar, V. (1922) Snoring disease in cattle. Madras Vet. Jl. No. 15, July 15th, 2-7.
- Krishnasamiengar(1896) Cattle disease of India.  
The Agricultural Ledger, No. 28.
- Lahaye, J. and Rulot (1926) Contribution de l'etude de l'hématurie chronique des bovidés.  
Ann. de. med. vet. 71, 192-207.
- Law, J. (1901) Haematuria. Vet. Med. Ithaca, 3, 205-207.
- Le blanc (1893) Sur d'hématurie des betes bovines. Bull. Soc. Centr. Med. Vet. = p. 86.
- (1899) Maladies de l'appareil urinaire in Cadeac's Pathologie interne des animaux domestiques. Paris. 7, p.88. Cited by Giovine.(1933).
- Lechlainche, E. (1936) Histoire de Medicine Veterinaire. Toulouse.
- Lienaux (1905) De la pathogenie et de l'etiology d'hématurie chronique des bovidés. Ann. de Med. Vet. 54, 185-198.
- (1919) Sur l'hématurie chronique des



- bovides. Ibid. 64, 329-350.
- Linder & Wust (1914) Assimilation of urea by yeasts and moulds. Chem. Abstracts, 8, 727.
- (1918) Ibid., 12, 1203.
- Lloyd (1923) Haematuria in a cow due to unusual lesion. Vet. Rec. 3 n.s., 502.
- Lockett, S. (1932) Red water of cattle in Jamaica. (A bladder disease). Jl. Jamaica Agri. Soc. 36, 16-19.
- Long, H.C. and Fenton, E.W. (1938) The Story of the Bracken Fern. Jl. Roy. Agri. Soc. Eng. 99, 15-36.
- Lucet, A. (1897) De L'Aspergillus fumigatus chez les animaux domestiques et dans les oeufs en incubation. Paris, p. 43, 47 and 73.
- Lynch, K.M. (1923) Aspergillus in scalp lesions following red bug (Leptus) bites. Arch. Derm. Syphil. 7, 599-602.
- MacKenzie, J.C. (1932) An economic survey of bovine Haematuria vesicalis in British Columbia: The Occurrence of the disease and results of a method of treatment. Thesis.Uni. Br. Columbia.
- Mallory F.B. & Wright J.H. (1924). Pathological Technique.
- Manca (1891) Studi storico-bibliografici

- interno all' ematuria dei bovini.  
Giorn. Med. Vet. Pract.   , p. 35.
- Mathis (1897) Sur les lesions et la patho-  
genie de l'hematurie essentielle des  
bovins. Jl. Med. Vet. et de Zoot.   ,  
p. 417.
- Matsumoto, T. (1929) The investigation of Aspergilli  
by serological methods. Trans. Brit.  
Myc. Soc. 14, 69-88.
- McKee, J.R. and McKee, C. (1928) Red water in cattle.  
Bull. Vancouver Med. Ass. 5, 55-69.
- Menard (1894) Bull. Soc. Centr. p. 381.  
Cited by Avril. (1929).
- Michin (1937) Blutiger Harn. Abst. Munch.  
Tierarztl. Woch.   , 176.
- Mickdahl, L.H. (1918) On the treatment of haematuria  
of cattle. Maandskr. f. Dyrl. 30, 582.  
Abst. Schweiz. Archiv. f. Tierhkl.  
1919, 61, 310.
- Miller (1891) Nephromycosis aspergillina.  
Austr. Med. J.
- Miyamoto, T. (1928) Urocystitis haemorrhagica of  
native cattle in Formosa. Jl. Jap.  
Soc. Vet. Sci. 7, No.3.
- (1927) Trans. Far East Ass. Trop. Med.  
Calcutta. 3, 667.
- Mosseray, Raoul (1934) Les Aspergillus de la section

- "Niger". Thom & Church. La Cellule, 43, Plate III, fig. 13.
- Moussu (1904) L'hématurie des bêtes bovines. Bull. Soc. Centr. Med. Vet. \_\_, p. 110 and p. 222.
- (1911) Traite des Maladies du Bétail. 3rd. ed. Paris.
- G. and Moussu, R. (1928) Cystite hémorragique. Hématurie des bovidés. Traite des maladies du gros bétail. 5th, edition. Paris.
- Mustak Hussain (1931) U.P. Vet. Mag. 19, No. 6, p. 4.
- Nicaud, P. (1928) Les formes actinomycosiques de l' Aspergillus fumigatus. Comp. rend. Soc. de Biol. 99 (33), 1565-7.
- Nicolaus, W. (1933) On the histogenesis and determination of the age of changes produced in fowls by Asp. fumigatus. Ztschft. f. Infekkrank. Haustier. 45, 191-209.
- Obici, A. (1898) Ueber die pathogenen Eigenschaften des Aspergillus fumigatus. Beitr. Path. Anat. Allg. Physiol. (Ziegler) 23, 197-237.
- Parameswara, Ayyar (1922) Madras Vet. J. 16th issue No. 4.
- Peck, S.M. (1930) Arch. Dermat. Syph., 22, 40.

- Pflug (1876) Die Krankheiten der uropoetischen systems der Haustiere. Wien.
- Pichon Cited by Giovine (1933).
- Piot Bey (1918) Bilharziosis as the cause of a perforating ulcer of the bladder in a calf. Rec. Med. Vet. 94, 631.
- Pommerret, M. (1937) Entamoeba Kamala et l'hématurie essentielle Dissertation. Paris. pp. 63.
- Popow, P.A. (1936) Chronic haematuria in cattle. Annu. Univ. Sofia. 9, 77-118.
- Racoonat (1847) Haematuria. Jl. Med. Vet. Ecole de Lyon. 3.
- Raistrick, Birkinshaw et al (1931) Studies in the biochemistry of microorganisms. Phil. Trans. Roy. Soc. Ldn. Series B, 222, 1-367.
- Rangaswamy (1922) Madras Vet. Jl. 14th Issue, No. 1.
- Records, E., Vawter, L.R. (1928) Red water disease of cattle. (Bacillary haemoglobinuria). Uni. Nevada Agri. Exp. Sta. Bull. No. 113.
- Reisinger (1924) Das Vorkommen von Stallrot der Rinder in Osterreich. Wien. Tier. Monatsch. 11, 39.
- Renon (1896) Passage du mycelium de l'Aspergillus fumigatus dans les urines au cours de l'aspergillose experimentale.



- Comp. rend. de sc. et memoire de la soc. biol. \_\_, April 18th.
- Reynal      Dictionnaire pratique de med. de chir. et de higylene veter. di Bouley et Reynal. Cited by Avril (1929) 9, p.10.
- Rivolta      (1891) Di una specie di ematuria (piscias angue) nei bovini. Il Mod. Zooiatro. p. 125.
- Rockwood, E.M. (1930) Arch. Derm. Syph. 22, 395.
- Roger      (1911) Contribution a l'etude de l'hematurie bovine. Rev. Vet. 36, 525-532, 593-599, 660-669.
- (1912) Bull. de la Soc. Centrale, 30? p. 329.
- (1916) L'oxalaemic des animaux domestiques. Bull. Soc. Centr. Med. Vet. 34, 468-78.
- Rosenow & (1922) Jl. Lab. Clin. Med. 7, 707-722.
- Ross      (1879) Blutharnen beim Rind. Tierarztl. Mitt. pp. 102-3.
- Rost, E.      (1926) Beitrage zur toxikologie der Oxalsaure und Oxalate. Jl. Pharm & Exp. Therap. 29, 257.
- Rothwell, Th. A. (1899) Aspergillois. M.D. Thesis. Victoria Uni. Manchester.
- (1901) Jl. Path. & Bact. 7, 34-52.
- Rudolf      (1928) Hamatologische studien bei

einigen Krankheiten des Rindes (including two cases of haematuria).

Deuts. tier. Woch. 36, 445-451.

Rutherford, J.G. (1911) Red water in British Columbia.

Ann. Rep. Vet. Dir. Gen. and Livestock Comm. Dept. Agri. Canada. pp. 39-40.

Sakamura, T. (1936) On some heavy metals for the cultivation of Aspergilli and the mode of eliminating traces of them from the culture medium. Jl. Fac. Sci.

Hokkaido Univ. Ser.V, Bot. IV. 3, pp. 99-116.

Salisbury (1875) Vegetations found in the blood of patients suffering from erysipelas. Zts. Parasitenk. 4, 1-5.

Sarvonat & Roubier (1911) Jl. Chem. Soc. 100, Pt.2, 815.

Savage, A. & Isa, J.M. (1932) Mycotic pneumonia of chickens. Sci. Agri. Canada. Vol. 13.

Sayers, R.R. & Meriwether, F.V. (1930) U.S. Pub. Health Rep. 45, 2994.

Scharer, R. (1930) Vorläufige Mitteilungen über die Rinder-Haematurie in Antioquia (Columbien). Schweiz. Arch. Tierhkl. 72, 475-477.

Schiemann, E. (1912) Mutationen beim Asp. niger. (Van Tieghem). Ztsft. Indukt. Abst.

- u. Vererb. 8, 1-35.
- Schlegel, M. (1912) Stallrot beim Rind. Zts.  
Tiermed. 16, 300.
- (1921) Haematuria vesicalis, Stallrot  
beim Rind. Zts. F. Infekt. Haust. 21,  
204-5
- (1934) Das Stallrot des Rindes.  
Munch. Tierarzt. Woch. 85, 365-371,  
380-384, 389-393, 404-408 and 416-421.  
Abst. Ind. Jl. Vet. Sc. Ani. Husb.  
1936, 6, 305-306 and 371-372.
- Shutt, F.T. and Robinson, C.H. (1930) Red water  
cattle disease as occurring in certain  
districts of British Columbia. Ann.  
Rept. Dir. Chem. Dept. Agri. Canada.  
pp. 107-112.
- Simonetti (1937) Abst. Vet. Bull. 1937, —, 376.
- Sinoir (1864) Cited by Giovine (1933).
- Smith, G. (1938) Industrial Mycology. Ldn.pp.
- Sonsino (1876) Cited by Neumann in Parasites  
and Parasitic diseases of domesticated  
animals. London. 1905, 532.
- Steyn (1933) Ond. Jl. Vet. Sc. Ani. Husb.  
1, 439.
- Stockfleth (1889) Handbuch der Tierarztlichen  
chirurgie. 2 teil, No.4. Leipzig.
- Stouki (1878) Haematuria in cattle. Vet.  
Jl. 6, 66.

- Takahashi, R. (1919) On the fungus flora of the soil. Ann. Phytopath. Soc. Japan. ==, pt. 2, 17-22.
- Talice, R.V. (1930) Le facteur pH en mycologie. Son influence sur la culture de certaines especes de champignons parasit-  
es de l'homme. Ann. Parasit. human. compar. 8, 183-188.
- Theiler, A. (1929) Health & Nutrition of Animals, p.44.
- Thom, C. & Church, M.B. (1921) Aspergillus flavus, A. oryzae and associated species. An. Jl. Bot. 8, 103-126.
- (1926) The Aspergilli, Baltimore.
- Topham, Thomas (1787) On the evacuation of blood urine, its causes and curative indications, demonstrated by a competent number of sections. A new compendious system on several diseases incidental to cattle. pp. 215-253.
- Turnstall, A.C. (1923) Micro-organisms associated with tea fermentations. Rev. App. Mycol.
- Unglas, G. (1932) L'argent colloidal irradié dans la cystite chronique bovine. Rev. de path. comp. et hygy. gen. p.461.
- Vaes (1843) Harnblasen ruptur beim Ochsen. Jl. Vet. de Belge , p. 182.



- Vet. Bull. (1931) A preliminary note on Chronic Bovine Haematuria (*Haematuria vesicalis*) in cattle. Vol.I. pp. 95-96.
- Vigney (1845)
- Wadia, D.N., Krishnan, M.S. & Makerjee, P.N. (1935) Geological foundations of the soils of India. Records Geo. Surv. India, 68, 363-391.
- Williams, T.H. (1894) Garden and field. 20, 244.
- Yakimoff (1926) Haematuria in the course of epizootics. Bull. Soc. Path. exot. 19, 686-688.
- Yoshimura, F. (1936) Action of copper and manganese upon the formation and colour of conidium on some species of *Aspergillus*. Jl. Fac. Sci. Hokkaido Univ. Bot.IV, 3. Series V, pp. 117-39.
- Yuill, E. (1939) Two new *Aspergillus* mutants. Jl. Bot. 77, 174-175.
- Zettler (1927) Zur aetiologie und Therapie des Stallrot (*Haematuria vesicalis*) der Rinder. Mitth. d. ver. Bad. Tierarz. 27 (Nos. 10 and 11) 85-88 and 94-96. Abst. Jahreb. Vet. Med. 47, 361.

Appendix

Statement showing cases of Haematuria occurring  
at the Wellington Farm from 1926 to 1936.

No.	Breed	Date of birth	Date of attack	Disposal of the animal
174	$\frac{3}{4}$ Ayr. Scindi	14-2-23	25-6-26	s. 3-9-27
24	H.B.Ayr. "	20-7-12	7-7-26	des. 8-10-26
181	$\frac{3}{4}$ Ayr. "	not known	10-7-26	d. 4-9-28
183	" " "	29-3-23	3-9-26	s. 29-8-29
117	Half bred Ayr. Scindi	8-6-17	9-12-26	d. 18-2-31
2	Ayrshire	5-4-21	16-4-27	d. 12-2-28
111	Half bred Ayr. Scindi	1-7-14	24-6-28	dis. 6-4-29
1	Ayrshire	18-4-21	9-1-29	d. 19-4-30
1	Half bred Ayr. Scindi	p. 18-9-28	24-6-29	d. 14-11-31
121	" " " "	1-5-18	24-6-29	s. 21-5-31
114	" " " "	2-4-17	7-8-29	s. 29-8-29
344	" " " "	22-2-27	12-9-29	d. 13-9-33
329	$\frac{3}{4}$ Ayr. Scindi	12-9-26	5-11-29	s. 17-6-30
116	Half bred Ayr. Scindi	25-5-17	26-12-29	s. 15-3-32
9	Ayrshire	10-4-25	7-1-30	d. 26-12-30
12	"	8-11-24	12-2-30	d. 16-1-31
20	"	6-3-26	24-2-30	d. 22-5-33
10	"	23-9-25	31-3-30	d. 31-1-31

s: sold      des: destroyed      dis: disposed of  
d: died      p: purchased

Statement (continued)

No.	Breed	Date of birth	Date of attack	Disposal of the animal
Bull Ideal	Ayrshire	1927	21-6-30	d. 21-11-31
6	"	6-4-25	23-6-30	d. 20-12-30
21	"	8-3-26	4-11-30	s. 15-3-32
22	"	12-10-25	16-11-30	s. 15-3-32
26	"	10-3-26	17-11-30	d. 23-1-31
Bull Farmer	"	24-6-26	19-12-30	d. 24-1-31
7	"	14-3-25	4-2-31	s. 15-3-32
381	Half bred Ayr. Scindi	9-10-28	11-3-31	d. Bangalore, date unknown
42	" " " "	2-11-14	6-6-31	s. 27-2-32
123	" " " "	5-11-18	13-7-31	s. 27-2-32
3	Ayrshire	15-5-26	27-11-31	s. 15-3-32
237	Half bred Hol. Harriana	2-4-25	13-1-32	s. 27-2-32
27	Ayrshire	23-1-29	28-1-32	d. 5-3-33
17	"	15-3-28	8-6-32	t. Bangalore, 11-3-34
3	$\frac{3}{4}$ Ayr. Jersey Scindi	p. 25-10-27	20-6-32	des. 10-12-32
34	Ayrshire	15-3-30	9-7-32	t. Bangalore, 11-3-34
199	$\frac{3}{4}$ Ayrshire Scindi	8-11-25	12-6-32	s. 15-3-33

d: died      des: destroyed      t: transferred to  
s: sold      p: purchased

Statement (continued)

No.	Breed	Date of birth	Date of attack	Disposal of the animal
Bullock 10	Country	not known	28-6-33	s. 22-7-35
" 147	$\frac{3}{4}$ Ayrshire	"	28-6-33	d. 7-6-34
58	Half bred Ayr. Scindi	12-3-27	11-7-33	at Wellington Farm
15	Country	12-3-27	7-11-35	at Wellington Farm
45	Half bred Ayr. Scindi	25-8-27	7-11-35	at Wellington Farm

s: sold      d: died

Note:-- The above animals suffered from haematuria with temporary relief at intervals of 15 to 20 days, otherwise the disease was continuous.



AppendixMETHODS.Staining Methods:-(I) Chromic acid-haematoxylin and light green.

- (1) Remove paraffin in Xylol, and Xylol in alcohol.
- (2) Allow 0.3-1% chromic acid to stand on the slide for about five minutes.
- (3) Wash in running water.
- (4) Stain with Mayer's haematoxylin for five minutes.
- (5) Wash in running water and pour on a few drops of tapwater substitute and allow to act for five minutes.
- (6) Wash again and stain for five minutes into 0.5% of aqueous light green.
- (7) Pass through ascending grades of alcohol for dehydration.
- (8) Clear in Xylol.
- (9) Mount in Canada balsam.

(II) Nicolle's Thionine and Orange G.

- (1) Stain section with Nicolle's Thionine for about five minutes.
- (2) Wash in water.
- (3) Differentiate in alcohol. (It does not take more than two minutes to complete differentiation)..
- (4) Wash in water.
- (5) Stain with 0.5 aqueous orange G. for one minute.

- (6) Pass quickly through ascending grades of alcohol.
- (7) Clear in Xylol.
- (8) Mount in Canada balsam.

(III) Claudius' method.

- (1) Stain section for about three to five minutes with 1% methyl violet solution.
- (2) Wash in tapwater.
- (3) Flood the section with half saturated solution of picric acid and allow it to stand for three minutes.
- (4) Wash in tapwater and dry section with blotting paper.
- (5) Decolourise in clove oil till there is no trace of methyl violet.
- (6) Clear in xylol.
- (7) Mount in Canada balsam.

(IV) Giemsa stain.

(Tissues for this should first be fixed in Bouin-Duboscq-Brasil or Schaudinn's solution).

- (1) Pour on 1% aqueous solution of potassium permanganate and let it stand for a few seconds. S
- (2) Wash in distilled water.
- (3) Remove the colour of potassium permanganate by pouring on a few drops of a 5% solution of oxalic acid. S
- (4) Wash thoroughly in changes of distilled water.
- (5) Stain with Giemsa for twelve hours (80 drops of

stock solution in 100 cc. of water. N.B. solution should be changed every day.

- (6) Wash in neutral distilled water.
- (7) Differentiate in 95% alcohol.
- (8) Clear in Toluol.
- (9) Mount in cedar wood oil.

(V) Feulgen's nuclear reaction.

Technique of the reaction -- three stages.

- (1) Hydrolysis effected by hydrochloric acid at 60° centigrade for about five minutes.
- (2) Slides transferred to fuchsin-sulphurous acid.
- (3) Excess stain removed by a thorough washing in water containing excess of SO<sub>2</sub>.

Hydrolysis sets free the aldehyde group of the thymo-nucleic acid when brought in contact with fuchsin sulphurous acid. This stains purple. Washing in sulphurous acid removes all the stain, the SO<sub>2</sub> preventing the formation of basic fuchsin from the fuchsin sulphurous acid. When therefore the sections are brought to water, preparatory to mounting, all the fuchsin sulphurous acid has been removed from the tissues, and what was previously chromalin is represented by a bright purple or violet colouration.

Preparations of Reagents.

- (a) Acid used for hydrolysis -- 82.5 cc. of HCl. (sp.

gr. 1.9) in 1000 c.c. of distilled water.

(b) Fuchsin sulphurous acid -- 200 c.c. of distilled water brought to boiling point. 1 gram of basic fuchsin is then added and the mixture well stirred. After the fuchsin has been dissolved, the solution is cooled to  $50^{\circ}\text{C}$ ., when it is filtered. The 20 c.c. of N.HCl. is added. When the liquid is further cooled down to  $25^{\circ}\text{C}$ ., one gram of anhydrous sodium bisulphite is dissolved in it. This results in the liberation of  $\text{SO}_2$ , and the fuchsin is slowly decolourised. It should be allowed to stand at least for twenty-four hours.

(c) Water containing excess of  $\text{SO}_2$ . To 200 c.c. of distilled water is added 10 c.c. of a 10% solution of anhydrous sodium bisulphite, and then 10 c.c. of N.HCl.. This water should be used so long as it gives a strong smell of  $\text{SO}_2$ . It must be renewed frequently.

#### Carrying out of reaction

(1) Fixation. Alcohol, alcohol acetic, sublimate or sublimate acetic can be used. Feulgen recommends sublimate acetic. Small pieces of tissues are fixed for twenty-four hours. Then washed in running water, brought up through alcohols to xylol and embedded in paraffin wax. Section cut at 5 microns. These are brought to water for hydrolysis. It is advisable to leave the sections in 90% alcohol before carrying out



the hydrolysis, as this hardens the tissues and reduces to a minimum the distorting action of the warm acid.

(2) Hydrolysis and colour reaction:- The acid used for hydrolysis must be kept at  $60^{\circ}\text{C}$ ., the other reagents are used at room temperature. For hydrolysis a beaker is used which is partly filled with acid and kept warm by a luminous Bunsen burner flame. It is essential to have a thermometer dipping into the acid. A slight fluctuation of a degree or so either way does not affect the result.

The tubes should be arranged thus:-

- (a) Tube I containing  $\text{HCl}$ ., one minute.
- (b) Beaker containing  $\text{HCl}$ . at  $60^{\circ}\text{C}$ ., four to fifteen minutes.
- (c) Tube II containing  $\text{HCl}$ ., few seconds.
- (d) Tube III containing distilled water, just rinse.
- (e) Tube IV containing fuchsin sulphurous acid,  $1\frac{1}{2}$  hours or more.
- (f) Tubes V, VI, VII, water containing excess of  $\text{SO}_2$ .  
Move slides from one to the other.
- (g) Tube VIII, distilled water, one to two minutes.

Length of time in fuchsin can be curtailed. Colouration of the chromatin is evident almost immediately and is intense after a few minutes. Contents of tubes V, VI and VII should be refilled when they no

longer give strong smell of  $\text{SO}_2$ .

Counter staining -- light green. Weak alcoholic solution after tube VIII. Dip in weak alcoholic solution of light green. (to 10 c.c. of distilled water add 1cc. of 1% alcoholic solution of light green).

Excess of stain washed in distilled water which is then blown off the slide.

Dehydrate and differentiate in absolute alcohol.

These are cleared in xylol and mounted in Canada balsam.

### Methods of Urine Examination

The following technique, based on the method described by Sheather (1923) was employed for the detection of protozoan cysts and helminthic ova in the urine of the affected and experimental animals. The material was obtained as fresh as possible.

- (1) Straining of the sample of urine, if necessary, through an ordinary muslin cloth.
- (2) A centrifuge tube was half filled with urine.
- (3) To this was added an equal quantity of sugar solution, prepared by dissolving 1 lb. of sugar in three quarters of a pint of water.
- (4) The tube was then rotated in an electric centrifuge machine for 2 minutes at a speed of 2000 revolutions per minute.
- (5) Both the top layer and the sediment at the bottom were then examined microscopically.

### Benzidine test for blood.

To the urine, which shows no visible blood colouration, add 2 or 3 c.c. of a mixture of equal parts of benzidine in absolute alcohol and hydrogen peroxide, a blue colour appears if blood is present. The enzyme peroxidase of the blood thus gives an indirect oxidase reaction on the addition of benzidine and the peroxide.

Appendix.

Experimental data of transmission work, as far as were available with the writer at Edinburgh are summarised below. Over forty bulls and calves were employed from time to time, and a number were still under observation with the writer's departure for Europe.

(a) Intravenously

(1) Hill Bull No.968, a healthy bull, aged three years, inoculated intravenously with 20 c.c. of a saline emulsion of urinary bladder growths from Hill Bull No.160 (haematuria) on 22-9-34. Apparently healthy throughout; urine and faeces examined for a few days with negative results. General condition continued fair. Gained about 70 lbs. in body weight.

Destroyed on 8-6-37. Postmortem apparently 'normal'.

(2) Hill Bull No.640, aged about 3 years, inoculated as above on 22-9-34 and yielded the same negative results. Blood smears on the first two days showed distinct anaemic changes. Gained about 50 lbs. in body weight. General condition continued fair throughout. Destroyed on 8-6-37. Postmortem 'normal'.

(b) Intra-cystically.

(3) Heifer Calf No.258, aged 1 year, inoculated on 22-9-34 directly into the emptied bladder with about 50 c.c. of a saline emulsion of bladder growths used above from Hill Bull No.160 (haematuria). Urine and faeces, negative. Gained about 50 lbs.. General con-



dition, fair. Other administrations given to this animal included: (1) On 11-4-35. Intracystically urinary sediment from Hill Bull No.1221. (2) On 17-3-36, 20 c.c. emulsion of a yellowish white fungus in N.S.S. administered orally and repeated every third day. The animal was alive and under observation till the writer left India. The animal being a female was not destroyed.

(c) Subcutaneously.

(4) Hill Bull No.143, 4 years, inoculated subcutaneously with 2 c.c. of a saline emulsion of washed urinary sediments obtained from Hill Bulls Nos.74 and 1221 (haematuria cases), maintained in plain broth and incubated for 24 hours at 37°C.. Inoculations given for about 4 months at intervals of 4 days, commencing on 6-10-34. Subjected to :- Subcutaneous injection of 2 c.c. of urinary sediment emulsion from Hill Bulls Nos.74 and 1221 on 6-10-34. Repeated the above every 4 days, 17 times from 11-10-34 to 2-1-35. Subcutaneous injection of 5 c.c. urinary sediment emulsion from Hill Bull No.428 (clinical case) on 16-10-35. Aspirated subcutaneously from the seat of above injection. Drenched about 20 c.c. of liver emulsion of Mouse No. 106.. Developed a few small abscesses from time to time, either disappearing intact in a few days, or healing off with a rather surprising rapidity after bursting or after being opened.(Compare with controls).

Pus smears were stained with Mann's stain (Dobell's modified technique for Amobae) etc., and examined. Gained about 75 lbs. in body weight. General condition was fair. Destroyed on 26-5-36. Postmortem examination carried out on 26-5-36 showed distinctly marked bladder disease, consisting of highly oedematous, nodular growths with patches of haemorrhagic lesions.

(5) Hill Bull No. 559. Inoculated subcutaneously 5 c.c. washed urinary sediments emulsion from Hill Bulls Nos. 74 and 1221 (haematuria cases) on 6-10-34. Died on 29-1-35. Temperature chart shows:- 3rd day after injection slight rise ( $1^{\circ}\text{C}.$ ) of temperature. Came down to normal before second injection. Rise of temperature,  $1^{\circ}\text{C}.$  transient. Temperature took a downward trend, went to subnormal. Rose again to  $103^{\circ}$  on the 9th day after the second injection. Developed an abscess on 8-11-34, opened spontaneously on 13-11-34. Pus smears examined. Gained about 70 lbs. in body weight steadily. Etiological diagnosis. Trachea and bronchi intensely haemorrhagic. Left lung a few echinococcus cysts. Blood smears negative. No lesions at the seat of inoculation. Kidneys and urinary bladder showed patches of haemorrhage.

#### Controls to (c)

These two animals were later transferred to another experiment.

(6) Hill Bull No.924. Subcutaneous injections of washed normal urinary sediments at 7 days' intervals for about three months.(Commencing on 11-10-34). Developed slight swellings, disappearing in a few days. The animal was healthy throughout, and its bodily condition was unchanged.

(7) Hill Bull No.1061. Given two injections of 5 and 10 c.c. of washed normal urinary sediment on 11-10-34 and 19-10-34. Slight thickenings at the seat of inoculations, disappearing soon.(Later subjected to other inoculations). Apparently healthy. Condition, fair. See below.

(d) Intrarectally.

(8) Hill Bull No.46, aged 7 years. Injected beneath the rectal mucosa with 3 c.c. of haematuria urinary sediments emulsion on 29-10-34. Soft faeces for 2 days about a week after the injection. No special dietetic cause was present. Apparently healthy. Gained about 60 lbs. in body weight. General condition, fair.

(9) Hill Bull No.193, aged 3 years. Same as above. Soft faeces for a few days about a week after the injections. Gained about 75 lbs. in body weight. General condition, fair.

Experimental Animals destroyed and Lesions Found.

(10) Hill Bull No.1061.

This Hill Bull was subjected to following inoculations:-

- 11-10-36 Injected subcutaneously 5 c.c. urinary sediment emulsion from a healthy bull.
- 19-10-36 Repeated the above injection with 10 c.c..
- 12-8-35 Injected 5 c.c. urinary sediment emulsion from Hill Bull No.74. (Clinical case).
- 22-10-35 Injected intravenously 20 c.c. urinary sediment culture from Hill Bull No.74.
- 22-10-35 Bled 1500 c.c. and 2000 c.c. respectively.  
&  
18-11-35 Destroyed on 29-6-36.

Lesions found:- Only a slight but well-established chronic congestion in patches seen in the bladder - no other abnormality.

(11) Hill Bull No.924.

Subjected to:-

- 11-10-34 Subcutaneous injection of 2 c.c. of urinary sediment emulsion from a healthy bull. This was repeated fifteen times at intervals of four days till 29-12-34. Subcutaneous injection of 2 c.c. of 24 hours' broth culture at 37°C. of urinary sediment emulsion from Hill Bull No.640 on either side of the neck on 16-10-35. Intravenous injection of 5 c.c. peritoneal matter emulsion of Mouse No.106. Destroyed on 11-6-36. Urinary bladder shows a few growths, rounded in appearance and smooth on surface. Mucous membrane shows old haemorrhagic discolouration.



(12) Hill Bull No.168.

Subjected to:-

30-10-35 Intravenous injection with 10 c.c. of heart emulsion of Mouse No.106. Destroyed on 22-6-36. Nothing abnormal could be found in the bladder, kidney and in the urinary tract.

(13) Hill Bull No.357. Subcutaneous injection with 10 c.c. of 24 hours' urine sediment culture in plain broth from Hill Bull No.428. The injection commenced on 30-7-36 and repeated at an interval of 4 days till 17-8-36 when Hill Bull No.428 died. Material for injection was obtained from Cow No.144, and injection is being continued at the same interval. The animal has up to the 5th October received a total of twenty injections. Blood passed in the urine on 11-9-36, and for a few days only subsequently.

(14) Hill Bull No.339 Control. Subcutaneous injection with 5 c.c. of 24 hours' urine sediment culture in plain broth from a healthy Hill Bull No.39. Injection commenced on 28-7-36 and repeated daily till 2-11-36. The animal was kept under observation till 1938 when it was destroyed, but no lesions were detected in the urinary organs.

(15) Hill Bull No.259. Injected subcutaneously with 5 c.c. of 24 hours' urinary sediment culture in plain broth from Hill Bull No.428. Injection commenced on 28-7-36 and continued every day till 18-8-36. The

The Hill Bull No.428 having died, material for injection was obtained from Cow No.144, and injection was continued on since then every day.

(16) Hill Bull 214. Injected subcutaneously with 5 c.c. of 24 hours' urinary sediment culture in plain broth from Cow No.2 on 27-7-36 and repeated daily till 2-11-36. Subcutaneous injection with 5 c.c. of 24 hours' urine sediment culture in plain broth from Cow No.2.. Injection commenced on 28-7-36 and continued since then every day.

(17) Hill Bull No.491. Transplanted a piece of haematuria bladder from Hill Bull No.74 behind the right shoulder joint on 17-6-36. Transplanted a piece of haematuria bladder from Hill Bull No.74 behind the shoulder joint in the right side on 17-6-36. The growth was found to be absorbed.

(18) Hill Bull No.385. Injected subcutaneously with 10c.c. of haematuria bladder emulsion from Hill Bull No.74 in the right chest on 17-6-36 and repeated on 9-7-36, 15-7-36, 20-7-36, 28-7-36, 30-7-36, 3-8-36, 6-8-36, 11-8-36 and 12-8-36. Subcutaneous injection 10 c.c. of haematuria bladder emulsion from Hill Bull No.74. The injection was started on 17-6-36, and continued with the same dose till 2-8-36, at an interval of about a week. From 6-8-36 the dose was increased to 20 c.c. and continued till the 13th August when it was stopped as the animal showed a rise of temperature.

from the 11th August to 13th August, 1936 injection with 20 c.c. of emulsion was given every day.

(19) Hill Bull No. 968. Injected intravenously with 20 c.c. of bladder growth emulsion in N.S.S. from Hill Bull No. 160 on 22-9-34.

(20) Hill Bull No. 640. Injected intravenously with 20 c.c. of bladder growth emulsion in N.S.S. from Hill Bull No. 160 on 22-9-34.

(21) Hill Bull No. 221. Transplanted subcutaneously a piece of papillomatous growth from the urinary bladder of Hill Bull No. 266 on 7-8-36. Transplanted subcutaneously a piece of papillomatous growth from urinary bladder of Hill Bull No. 266 on 7-8-36. There was no abscess formation.

(22) Hill Bull No. 341.

(a) Injected subcutaneously with 10 c.c. of papillomatous growth from urinary bladder of Hill Bull No. 266 on 7-8-36 and repeated on 10-8-36, 11-8-36, 12-8-36, 13-8-36, 14-8-36, 16-8-36 and 17-8-36.

(b) Injected subcutaneously with about 5 c.c. of emulsion or urinary sediment in normal saline solution, showing trypanosomes from Hill Bull No. 428 on 18-8-36.

(c) Injected subcutaneously 10 c.c. of emulsion of the urinary bladder growth from Hill Bull No. 428 on 20-8-36 and repeated the same on 21-8-36, 22-8-36, 23-8-36 and 24-8-36. Subcutaneous injection with 10 c.c. of papillomatous growth emulsion from urinary

bladder of Hill Bull No.266. Injection commenced on 7-2-36, and in all eight injections were given up till 17-8-36. Four more injections in the same doses were given, from the urinary bladder emulsion of Hill Bull No.428. This animal was further subjected to a subcutaneous injection of about 5 c.c. of urinary deposit in normal saline from Hill Bull No.428, showing living trypanosomes in the urine.

(23) Hill Bull No.877.

(a) Injected intraperitoneally 5 c.c. of papillomatous growth emulsion from urinary bladder of Hill Bull No.266 on 7-8-36.

(b) Injected intraperitoneally 10 c.c. of papillomatous growth emulsion from Hill Bull No.428 on 18-8-36.

(24) Hill Bull No.879. Control.

Injected subcutaneously with 10 c.c. of 24 hours' healthy urinary sediment culture on 27-7-36 and repeated the above injection every third day till 27-10-36.

(25) Hill Bull No.497. Injected subcutaneously with 10 c.c. of 24 hours' urinary sediment culture from Cow No.2 on 27-7-36 and repeated at intervals of 3 days till 29-10-36. Subcutaneous injection with 10 c.c. of 24 hours' urinary sediment culture in plain broth from Cow No.2. Injections repeated at an interval of 4 days. The animal has received up till 5th october, 1936, a total of twenty injections.

(26) Hill Bull No.576. Inoculated intravenously 30



c.c. of an emulsion of kidney from Rabbit No.9 (died of fungus inoculation) on 5-5-38. Repeated 50 c.c. of an emulsion of kidney, liver, spleen, lung etc. of Rabbit No.60 on 15-6-38. (Slight protein shock was noticed). Urine negative for haematuria, but there was an increase in the cellular content after the injection.

(27) Hill Bull No.483. Intravenously about 50 c.c. of a saline emulsion of lichen collected from the local vegetation on 29-6-38. There was only a slight rise of temperature following the injection (to 102.4). Marked abdominal breathing, associated with slight salivation and attempt to defecation was noticed. Urine negative for haematuria. Slight increase of cellular contents.

(28) Hill Bull No.220. Administered per os about  $\frac{1}{2}$  ounce of lichen (by weight) on 29-6-38, 1-7-38, 3-7-38, and 5-7-38. No significant change noticed.

(29) Hill Bull No.279. Control

Subcutaneous injection with 10 c.c. of 24 hours' urinary sediment culture in plain broth from a healthy Bull No.39. Injections are given at an interval of 4 days, and the animal has received in all twenty injections.

Appendix.Aspergillus Culture given to Small Animals.Rabbits.

- (1) R. No.40, inoculated subcutaneously 0.5 c.c. of emulsion of culture from Gaiety on 16-7-38. Died on 22-1-38. P.M. Rupture of the left auricle.
- (2) R. No.41, inoculated subcutaneously with 0.5 c.c. of emulsion of culture from Gaiety on 16-7-38. Died on 20-1-38. P.M. Killed by a wild cat.
- (3) R. No.181, inoculated intravenously 4 c.c. of emulsion of fungus culture from Gaiety on 27-4-38. Died 30-4-38. P.M. Lungs congested, heart congested, liver enlarged and congested with white specks. Kidney haemorrhage. Bladder vessels engorged. Heart blood gave pure culture of Aspergillus. (Aspergillosis)
- (4) R. No.9, inoculated intravenously with 4 c.c. of emulsion of fungus (Gaiety) on 27-4-38. Died 30-4-38. P.M. Typical lesions. Aspergillosis.
- (5) R. No.159, intravenously with 1 c.c. of a saline emulsion of 48 hours' old fungus culture from Hill Bull No.143 on 16-5-38. Died 17-5-38. P.M. Typical lesions of aspergillosis.
- (6) R. No.14, intravenously one c.c. of a saline emulsion of fungus isolated from a haematuria case. (Gaiety) 30-4-38. Died on 4-5-38. P.M. Lesions of Asper..
- (7) R. No.15, intravenously one c.c. of a saline emulsion of fungus isolated from a haematuria case, Gai-

- ety, on 30-4-38. Died on 5-5-38. P.M. Lesions of Asp..
- (8) R. No.20, intravenously one c.c. of a saline emulsion of 48 hours' old fungus culture from Hill Bull No.549(O.K.)on 16-5-38. Died on 17-5-38. P.M. Aspergillosis (lesions not well developed).
- (9) R. No.19, intravenously one c.c. of a 48 hours' old fungus culture in saline on 16-5-38. (Isolated from Cow 4/26) Died on 18-5-38. P.M. Aspergillosis (lesions not markedly developed).
- (10) R. No.232, intravenously one c.c. of a 48 hours old fungus culture isolated from (Lichen) in N.S.S. on 20-5-38. Bladder vessels dilated and engorged. Kidneys haemorrhagic spots. Lungs petechiated. Liver enlarged. Died on 22-5-38. P.M. Aspergillosis.
- (11) R. No.233, intravenously one c.c. of a saline emulsion of 48 hours' old fungus culture isolated from a local vegetation (fern) on 20-5-38. Both lungs oedematous, petechiated and highly congested. Liver enlarged and congested. Kidneys enlarged and highly haemorrhagic. Bladder, nothing unusual. Caecum haemorrhagic. Died on 23-5-38. P.M. Aspergillosis.
- (12) R. No.47, inoculated intravenously one c.c. of a saline emulsion of greenish material sticking to the twig (lichen) collected from the local vegetation on 31-5-38. Kidneys highly haemorrhagic lesions. Urinary bladder contained haemorrhagic urine. Died on 2-6-38. P.M. Typical lesions of Aspergillosis.

(13) R. No.30, intravenously about 2 c.c. of a saline emulsion of kidney of R. No.47 on 3-6-38. Died on 3-6-38. E.D. -Anaphylactic shock.

(14) R. No.60, intravenously about 1 c.c. of an emulsion of fungus isolated. Lungs elevated nodules and haemorrhages. Liver enlarged and congested. Died on 15-6-38. E.D. Aspergillosis (typical lesions).

#### Mice.

(15) M. No.45, inoculated subcutaneously with 0.25 c.c. of emulsion of culture "Gaiety" on 16-7-37. Died on 8-11-37. Postmortem. No naked eye lesions seen.

(16) M. No.46, inoculated subcutaneously 0.25 c.c. of emulsion of culture from Gaiety on 16-7-37. Died on 3-11-37. Postmortem. Pneumonia.

#### Guineapigs.

(17) G. No.228, inoculated subcutaneously about 3 c.c. of an emulsion of fungus isolated from "Gaiety" on 27-4-38. Millet seed sized lesions on liver, spleen enlarged with blood. Bladder, haemorrhages on mucous membrane. Kidney congested. Died on 4-6-38. E.D. Aspergillosis.

(18) G. No.180, Inoculated subcutaneously about 3 c.c. of an emulsion of fungus isolated from "Gaiety" on 27-4-38. Died on 13-6-38. Kidneys showed raised white speck-like lesion. From heart blood and kidney the fungus was isolated in pure culture. E.D. Aspergillosis.



Appendix.Experimental Calves.Urinary Sediments.

## (1) Heifer Calf No.30.

Urinary sediment in normal salt solution administered by the mouth every three or four days. Urine was positive for haematuria on several occasions for four months and showed an increase in the cellular contents of the urine on many occasions. Observations discontinued with writer's departure.

## (2) Bull Calf No.154.

5 c.c.of urinary sediment in normal saline from Cow No.4/26 given subcutaneously on 26-12-37 and repeated on 5-1-38. The animal died on 11-1-38. Autopsy showed highly extensive, and intense haemorrhagic inflammation in the subcutaneous tissues at the seat of injection. The *Aspergillus* isolated from the heart blood. Death due to rapid diffusion of the fungus into the general system.

Aspergillus Cultures.

## (1) Heifer Calf No.38.

5 c.c. of an emulsion of the pure culture in normal saline, culture originally isolated from "Gaiety", given subcutaneously on 26-12-37, and repeated at four day intervals on seventeen occasions. The animal showed very slight rises of temperature after each inoculation. For the last six days of its life it showed a continued subnormal temperature below 98°C.. Cultural

examination of the heart blood and internal organs failed to reveal any bacterial organism of significance. The fungus was isolated from the heart blood, the kidney and the cutaneous lesion. Histological sections of the visceral lesions confirmed their fungal origin. Marked lesions were present in the urinary tract and the liver.

(2) Heifer Calf No.34.

5 c.c of an emulsion of fungus from "Gaiety" at an interval of a fortnight intravenously. Twelve such injections given. Urine negative for haematuria but the cellular content of the urine was very considerably increased. With the writer's departure the animal was discontinued from this experiment and transferred to Dairy on 17-5-38.

(3) Heifer Calf No.258.

Intravenously about 6 c.c. of a thin emulsion of fungus isolated from "Gaiety" on 3-5-38. Repeated 10 c.c. on 25-5-38. Cellular contents of the urine considerably increased. No actual haematuria exhibited till the writer's departure for Europe.

Adult Cattle.Aspergillus CultureHill Bull No.255.

Inoculated intravenously with pure culture A.flavus series, recovered from the clinical case "Cow Gaiety", 50 c.c. of a thin emulsion in normal saline being given. The inoculation was made on 5-5-38 and the animal was destroyed on 4-6-38, when in the moribund condition. The tissues were collected warm and fresh. After the initial inoculation the temperature rose to about 105°C. for two or three days and then gradually came down to normal. For the last three days or so of its life a subnormal temperature was recorded. The kidneys showed contractions and atrophy of lobules. The surface showed numerous depressions and marked greyish patches of characteristic lesions. The kidney tissue proper was oedematous and highly tense. A cyst in the kidney was found to contain a pure culture of the fungus. The urinary bladder contained red flakes, while the organ showed a number of oedematous nodules of varying sizes. The submucous haemorrhages were quite prominent. Spleen was intensely congested but otherwise nothing unusual could be discerned. Liver was abnormal in colour and fatty in consistence. Numerous haemorrhagic and greyish lesions were present in this organ. The lungs and the air sinuses showed typical lesions.